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SELENIUM POISONING[1]

ALVIN L. MOXON AND MORRIS RHIAN

Experiment Station Chemistry Department, South Dakota State College, Brookings, S. D.

The element selenium was discovered by Berzelius and Gahn in 1817 while they were examining the sediment from a sulfuric acid plant at Gripsholm, Sweden. Although the total production of selenium has never been very large, it has several important industrial uses, especially in the chemical, electrical, ceramic and metallurgical industries. The yearly world production of selenium in peace time is about 1,000,000 pounds, most of which is recovered from the anode slimes of copper refineries. Waitkins, Bearse and Shutt (180) have recently presented an excellent review on the commercial importance of selenium.

Selenium compounds are of importance in carrying out certain chemical reactions. Selenium has been useful as a dehydrogenating agent in determining the structure of complex organic compounds (137). It has also been used as a specific oxidant (145) and as a reagent for ascorbic acid (89).

The greatest interest in selenium and its compounds has been aroused in relation to its toxic effects in the animal body. Although Japha (70)[2] proved as early as 1842 that selenium was definitely toxic, it was not associated with general livestock poisoning ("alkali disease") until 1931.

Probably the first authentic written record of selenium poisoning ("alkali disease") in livestock is the report written in 1856 by Madison (92), an Army surgeon stationed at Fort Randall, territory of Nebraska.[3] He described a fatal disease among the horses which had grazed in a certain area near the fort. The horses lost the long hair from the mane and tail, and their feet became so sore that they were unable to move about in search of food.

Soon after the settlement of the territory around Fort Randall, about 1891, the farmers experienced difficulty with the same disease that Madison had described. They called it the "alkali disease" because they associated it with alkali seeps and waters of high salt content. Even though experiments carried out at the South Dakota Experiment Station as early as 1912 and 1913 (83) (84) proved that suspected water did not cause the malady, the name "alkali disease" is still associated with the livestock disturbance which is now known to be chronic selenium poisoning.

In 1929 Franke started his investigations at the South Dakota Experiment Station which led to co-operative work with several bureaus of the U. S. Depart-

[1] Approved for publication by the Director of the South Dakota Agricultural Experiment Station.

[2] Jones (Biochem. J. **4**: 405, 1909) has credited Gmelin (54) with the first work on the effect of selenium on the animal body. The reviewers have examined Gmelin's book and have failed to find any mention of selenium.

[3] Part of the chapel at Fort Randall is still standing on its original site in southeastern Gregory county, South Dakota, just three or four miles from the Nebraska boundary.

ment of Agriculture and the discovery, by Robinson (146), of selenium in cereals which Franke had found to be toxic to laboratory animals. A more detailed account of the early work on the selenium problem has been published (103). Reviews on certain phases of the selenium problem have appeared at various times since the discovery of selenium in toxic grains (29) (60) (93) (97) (129) (154) (169) (173) (174) (180) (185). The selenium problem as it is now recognized may be summarized briefly as follows: Soils which have been derived from certain geological formations contain selenium which is available to plants and is absorbed by the plants to the extent that they are toxic when consumed by animals. This toxic vegetation causes considerable livestock losses and is, directly and indirectly, a hazard to public health in certain areas.

In this review the present status of the selenium poisoning problem will be discussed under the main divisions of: Geological Distribution of Selenium, Selenium in Soils, Absorption of Selenium by Plants, The Use of Selenium for the Control of Insects on Plants, Selenium Poisoning in Livestock, Selenium Poisoning in Poultry, Experimental Selenium Intoxication in Laboratory Animals, Organic Selenium Compounds, Public Health, and Methods of Analysis for Selenium.

The Geological Distribution of Selenium. Franke et al. (49) reported the observation that the cases of "alkali disease" of livestock encountered in their preliminary survey all occurred on "gumbo" soils derived from Pierre shales. Beath and co-workers (7) were the first to work on the geological distribution of selenium in relation to the poisoning of livestock. They had associated poisonous plants with Cretaceous shales before they found selenium in the plants. Many of these plants grow either on the unweathered shale or soils derived from the shale.

Cretaceous formations have contributed most of the selenium for the formation of soils which produce toxic vegetation (8) (9) (14) (15) (19) (103) (113) (114). In South Dakota the Cretaceous formations have been mapped, sampled and analyzed for selenium (113) (114) and with the data thus obtained it is possible to predict, in some detail, areas which are capable of producing toxic vegetation. Stratigraphically the parent material which has produced most of the seleniferous soils in South Dakota (113) (114) (152) occurs near the top of the Pierre formation in the Mobridge member, at the base of the Pierre in the Sharron Springs member, and in the upper (Smoky Hill) member of the Niobrara formation which lies just below the Sharron Springs member of the Pierre formation.

Highly seleniferous soils have been derived from the Mobridge member of the Pierre formation in northern Nebraska and southern South Dakota. Because of the stratigraphic position of this member of the Pierre formation it has been the parent material for soils over large areas. Fortunately, the selenium content of the soils is high only in the areas adjacent to the Missouri River in the south central part of South Dakota and in north central Nebraska (114). To the west and north the selenium content of the Mobridge and the derived soils decreases to unimportant amounts. Soils which have been derived from the Mobridge member are of more value for crop production than the soils which have been

derived from any of the other members of the Pierre formation. Many of the soils derived from the other members are not suitable for cultivation and are used only for grazing purposes.

The Sharron Springs member at the base of the Pierre formation contains considerable quantities of selenium in South Dakota, but its outcrops are limited to small areas and it does not weather readily. Therefore, very little productive soil has been derived from it in this area.

The upper part of the Niobrara formation (Smoky Hill member) appears to be seleniferous and capable of producing toxic vegetation wherever it outcrops or occurs near the surface. Some of the most seleniferous soils in western South Dakota (113) (114), Wyoming (14) (15) (74), Kansas (15) and Colorado (14) (15) have been derived from the Smoky Hill member of the Niobrara formation.

Selenium has been found in formations older than the Cretaceous formations, but in most cases it appears to be present in amounts insufficient to produce highly toxic soils or vegetation. Beath, Gilbert and Eppson (10) reported that formations of Permian and Triassic age contain selenium in Wyoming and Idaho.

Williams, Lakin and Byers have reported that glacial drift contains selenium in North Dakota (190) and in Canada (190) (18). During the past year a detailed study has been made of a glaciated area in South Dakota on which selenium "indicator" plants have been found (164). It was revealed that the selenium in this glacial drift occurs largely in glacial lake silts.

Small amounts of selenium are continually being removed from seleniferous soils by leaching. The Colorado River carries appreciable quantities of selenium below irrigated areas where drainage and seep water come from seleniferous soils (186). This selenium is carried to the Gulf of California. Selenium has been found in deposits from the bottom of the Gulf of Mexico (114) and in the sea-bottom deposits from various parts of the world (187) (15). The fact that ocean water contains no more than a trace of selenium while marine deposits, both ancient (Cretaceous formations) and modern, contain selenium in appreciable quantities indicates that the element is precipitated upon reaching the ocean. The mechanism for the precipitation is suggested by the work of Strock (169), Byers et al. (19) and Olson and Jensen (124) who found that selenite selenium is rapidly precipitated from solution in the presence of colloidal ferric hydroxide.

Selenium has been found in small amounts in some meteorites from various parts of the world (17) and in rather large amounts in volcanic lavas from Hawaii (20).

Selenium in Soils. Moxon, Olson and Searight (114) have estimated that from 60 per cent to 80 per cent of the selenium in the original geological formation is lost in the soil forming processes. Thus, soils containing two to four parts per million of selenium have been formed from geological formations which originally contained about ten parts per million of selenium. The balance of the selenium is lost through leaching during the soil forming processes.

Much of the work which has been done in locating seleniferous soils has been

of a reconnaissance nature. Geological formations which are known to be seleniferous and "indicator" plants (11) have been of great value in these surveys. Byers and co-workers (14) (15) (19) (77) (189) (190) have made the most extensive surveys which have covered large areas in the United States and certain areas in Mexico (16) and Canada (18) (190). In much of the reconnaissance work only samples of surface soil have been taken for analysis, but the intensive field studies of Olson et al. (125) (126) (128) indicate that the selenium content of the surface soil is of little value in predicting the capability of the soil to produce toxic vegetation. The importance of sampling technique in survey work has been pointed out by Olson, Whitehead and Moxon (128). Samples of soils and vegetation taken at two hundred foot intervals showed extremely wide variations in selenium content.

Byers and co-workers (19) (188) have reported that selenium occurs in soils in the following forms: *a*, elemental; *b*, pyritic or selenide; *c*, selenite; *d*, selenate, and *e*, organic. Olson and Moxon (127) found that the humus of some surface soils contained up to 40 per cent of the total selenium. However, recent detailed field studies have shown that the selenium (organic and inorganic) content of the surface soil is of little importance as a source of selenium for plants. The water soluble selenates in the second and third feet of the profile are the important source of available selenium for grass plants. Some selenite was found in this water solution (128).

Lakin, Williams and Byers (79) have shown that there are large areas of highly seleniferous soils in Hawaii and Puerto Rico which do not produce toxic vegetation. They concluded that the selenium in these "non-toxic" soils occurs largely as a basic iron selenite which is not available to plants. Moxon, Olson and Searight (114) found that basic iron selenite was not readily available to plants in greenhouse experiments.

Hurd-Karrer (65) (66) and Byers (14) from greenhouse experiments have reported that the sulfate content of soils has a great influence on the absorption of selenium by plants. In experiments carried out by the South Dakota Experiment Station (43) on field plots in a seleniferous area, the addition of sulfur failed to inhibit the absorption of selenium by crop plants. Hurd-Karrer (68) observed that sulfates would protect plants against selenates but not against selenites. Selenates constitute the main source of selenium available to plants at least in some highly seleniferous soils (128) but since the available selenium occurs in the second and third feet of the soil profile, it would be difficult to mix sulfates or other forms of sulfur into the soil thoroughly enough to be of much practical value. Furthermore, the seleniferous soils usually contain high concentrations of sulfates.

Absorption of Selenium by Plants. Cameron (21) appears to be the first to have demonstrated that plants would absorb selenium from soils to which it had been added. He reported his work in 1880. In 1885 Knop (75) demonstrated that selenic and selenious acids are absorbed by plants. In 1934 Beath et al. (6) reported that certain plants could be used as "indicators" for seleniferous soils and geological formations. These "indicator" plants are all classified

in the genera: *Stanleya, Oonopsis, Xylorrhiza* and *Astragalus* (certain species). Field observations (8) (9) (19) (103) suggested that selenium was essential for the growth of the "indicator" plants, and Trelease and Trelease (176) have reported that selenium is a stimulating if not an essential, element for "indicator" plants. They have also studied physiological differences of "indicator" species, and "non-indicator" species of *Astragalus* (177). Trelease (172) has classified a number of species of Astragalus by germination tests in the presence and absence of sodium selenite. Beath, Gilbert and Eppson (11) have recently summarized their extensive work with "indicator" plants.

In range areas the highly seleniferous "indicator" plants are of importance as a livestock hazard. In seleniferous farming areas, however, the "indicator" plants are usually not as abundant as they are in grazing areas, and because of the nature of the associated vegetation they are not especially important as a livestock hazard. Crop plants and grasses are sources of selenium causing livestock losses in farming areas. While the selenium content of "indicator" plants often reaches several thousand parts per million, the selenium content of cereals is seldom as much as one hundred parts per million.

There has been no satisfactory explanation for the ability of "indicator" plants to absorb such large amounts of selenium under conditions where ordinary plants absorb only traces of the element. This, certainly, should be a challenging problem for plant physiologists and plant biochemists.

Robinson (147) determined the selenium content of wheat from various parts of the world. A sample from South Africa contained 1.5 p.p.m.[4] which was the highest amount found in imported samples, except for a sample from Saskatchewan, Canada which contained 1.9 p.p.m. Samples of wheat collected from seleniferous areas in South Dakota contain up to 30 p.p.m. of selenium and one sample of wheat which was grown on soils derived from the Niobrara formation has been found to contain 63 p.p.m. of selenium. This is the highest concentration of selenium in any sample of field-grown wheat collected from seleniferous areas. Hurd-Karrer (67) produced wheat containing 220.0 p.p.m. of selenium by adding sodium selenate to soil in the greenhouse. The sample of wheat which contained 63 p.p.m. of selenium was milled in an experimental mill (115) and the fractions analyzed for selenium. The bran contained 88.44 p.p.m., the shorts 77.18 p.p.m. and the patent flour 53.26 p.p.m. of selenium. The percentage distribution of selenium in the fractions of this milled sample was comparable to the distribution of selenium in three other samples of wheat, of lower selenium content, which were milled and analyzed at the same time.

Thorvaldson and Johnson (171) of the University of Saskatchewan analyzed 230 composite samples representing 2,230 individual samples of wheat, mainly from the 1938 crop, grown in the province of Saskatchewan. The maximum selenium content of the composites was 1.5 p.p.m. Individual samples were found with a maximum of 4.0 p.p.m. selenium.

Byers and Lakin (18) analyzed a number of "indicator" plants and a few miscellaneous plant samples from the provinces of Alberta, Saskatchewan and

[4] p.p.m. = parts per million.

Manitoba, Canada. One sample of young wheat plants from Saskatchewan contained 120 p.p.m. of selenium; another sample contained 30 p.p.m.

Lakin and Byers (78) determined the selenium content of 951 samples of wheat and wheat products from Minnesota, North Dakota, South Dakota, Nebraska, Kansas, Colorado, Wyoming and Montana. Slightly more than 30 per cent of the samples collected in South Dakota contained over 4.0 p.p.m. of selenium. Five per cent of the samples from Kansas contained over 4.0 p.p.m. of selenium. It should be pointed out that their samples are by no means representative of wheat produced in states where seleniferous areas have been mapped, because they selected most of their samples in areas known to be highly seleniferous. If their samples had been picked at random like the samples collected in Canada (171) the average selenium content would have been much lower. This might well be illustrated by the values obtained by Moxon and Sebesta (119). They analyzed 86 samples of wheat grown in 1939 which were taken at random from 14 counties in south central South Dakota. Seventy-two of these samples contained less than 1.0 p.p.m. of selenium. Only one sample contained over 4.0 p.p.m., and it contained only 5.0 p.p.m. It would have been possible to select samples with higher concentrations of selenium. Likewise, it should be possible to select individual samples high in selenium content in other states or in Canada after a study is made of the occurrence of selenium on many individual farms as has been done in South Dakota.

Olson, Jornlin and Moxon (126) determined the selenium content of several grasses and plants collected over a three-year period from fenced plots on a highly seleniferous ranch. The results indicated that grasses containing from 10 p.p.m. to 20 p.p.m. of selenium will produce typical symptoms of chronic selenium poisoning ("alkali disease") in cattle.

Perkins and King (136) found that selenium as sodium selenite when applied at rates up to 2.5 p.p.m. of the soil in greenhouse experiments stimulated germination, spring growth and harvest weight of Tenmarq wheat. Applications of selenium greater than 8.0 p.p.m. killed the wheat. Levine (87) and Stanford and Olson (165) have also observed that selenium in small amounts stimulated the growth of plants. For wheat plants grown in culture solutions (68), selenite selenium was more toxic than selenate selenium in the presence of high concentrations of sulfur while the toxicities were reversed if the sulfur concentration was below 30 p.p.m.

Painter and Franke (132) have studied the selenium sulfur relationships in cereal plants and concluded that although the metabolism of selenium and sulfur is similar in cereal plants, it is not identical for these two elements.

The Use of Selenium for the Control of Insects on Plants. The toxicity of selenium to insects has led to the use of some selenium compounds as control measures. Sprays for the control of insects on citrus fruits have been prepared by dissolving selenium in a solution of potassium ammonium sulfide (55). The United States Department of Agriculture in 1933 issued a warning regarding the use of selenium-containing spray materials, because little was known about the effects of the residues of such sprays (123). In the same year the California

Agricultural Experiment Station began a study of the use of selenium-containing sprays on citrus fruits and grapes. In 1938 the results of this study were reported by Hoskins, Boyce and Lamiman (64). The selenium-containing sprays were effective for the control of mites on citrus and grapes. Based on the limited information available at that time, they concluded that if the selenium sprays were properly used there would be no danger to public health, in spite of the relatively high selenium content of the grapes.

Hurd-Karrer and Poos (69) found that aphids are killed by concentrations of selenium in wheat plants that are too low to cause injury to the plants. Experiments carried out in Trinidad (138) have shown that cotton plants can be rendered toxic to the cotton stainer and the pink bollworm by the application of small amounts of sodium selenate to the soil. Neiswander and Morris (122) have shown that the application of small amounts of selenium to soils will control red spiders on ornamental plants. They observed significant reduction in both mites and other insects on plants which contained selenium.

On the basis of present information, the application of selenium to soils as a control measure for insects should be discouraged. The concentrations of selenium applied are such that many plants could be made distinctly toxic.

Even though some insects can be killed by adding selenium to the soil or by spraying the plants with selenium compounds, there are certain insects which thrive on highly seleniferous plants and seeds. Trelease and Trelease (175) found weevils (*Acanthoscelides fraterculus*) and seed-chalids (*Bruchaphogus mexicanus*) completing their life cycles in seeds of *Astragalus bisulcatus*. The seeds contained 1,475 p.p.m. of selenium. Byers et al. (19) found flies (possibly *Pseudotephritis*) living on a sample of *Astragalus racemosus* plants which contained 1,800 p.p.m. of selenium. Moxon (105) analyzed grasshoppers (*Melanoplus bivittatus*) which were collected from seleniferous plants. They contained as much as 20 p.p.m. of selenium and exhibited no deleterious effects from the seleniferous diet.

Selenium Poisoning in Livestock. Selenium poisoning of livestock has been divided into two general classes, chronic and acute or sub-acute (29) (103). The chronic type, "alkali disease," is predominant in South Dakota and in other states where seleniferous soils are farmed extensively. It results from the consumption of vegetation, grains and forages containing up to approximately 25.0 p.p.m. of selenium for a period of several days or weeks. The acute type, "blind staggers," is the common type in Wyoming and in the range areas of other states where highly seleniferous "indicator" plants are abundant. These plants usually contain several thousand parts per million of selenium and frequently cause death within a short time when consumed in relatively small amounts by livestock.

Draize and Beath (29) and Beath et al. (7) (8) have described and discussed the acute type of poisoning, "blind staggers," in considerable detail. The term "blind staggers" is a rather misleading designation because the animals may not become blind, and they may not stagger about in all cases. In the early stages the animal may stray from the herd; there is usually a slight impairment of vision and the animal has difficulty in judging nearness of objects in its path.

In the next stage the blindness usually becomes more pronounced and is accompanied in most cases by a depraved appetite and the desire to chew wood, bone, metal objects, etc. There is also a greater tendency to wander, often aimlessly, in circles, and in case a solid object is encountered, the animal makes an effort to push forward rather than to turn to one side and go around the object. The last stage is characterized by various degrees of paralysis. There is evidence of abdominal pain, and death results from failure of respiration, much the same as has been observed in dogs and rats which have been injected with lethal doses of selenium (3) (39). Some of the symptoms observed by Miller and Williams (98) in horses, mules and cattle which had been subjected to lethal doses of sodium selenite were similar to the symptoms of "blind staggers."

Symptoms of the "alkali disease" or chronic selenium poisoning have been observed in a number of "alkalied" animals (29) (103). Dullness and lack of vitality is a general symptom. The animals become emaciated, stiff and lame, and fail to respond to good care and selenium-free feed. A prominent symptom in horses and mules is the loss of the long hair from the mane and tail. For this reason the chronic form of poisoning is often referred to as the "bobtailed disease." This loss of hair from the mane and tail often takes place within a month after horses are moved to seleniferous areas. Madison (92) mentioned the fact that symptoms of the "alkali disease" appeared in dragoon (cavalry) horses brought to Fort Randall just ten days after they arrived from Fort Lookout and from Big Sioux River (eastern South Dakota). Of special interest to the reviewers is the fact that the horses arrived at Fort Randall (from non-seleniferous areas) on August 10, since it has been our observation that symptoms of "alkali disease" usually start to appear at about this time of year in animals on seleniferous ranges. The appearance of the symptoms usually follows very closely the occurrence of hot winds which dry the green vegetation.

The loss of long hair from the mane and tail in horses and mules, and the loss of the long hair from the switch of cattle is usually accompanied or followed shortly by lameness and soreness of the feet. Swelling appears at the coronary band. In very mild cases there may be no further change although the animals may be lame for some time. In severe cases a gradual separation of the wall of the hoof occurs below the coronary band, and a new growth of hoof starts at the coronary band. In some cases the old hoof is sloughed off and in others it remains attached to the new growth until the new hoof has grown to normal length. In cattle the old hoof usually remains attached to the new growth and in cases where there have been several attacks the old ragged hoofs may be 8 or 10 inches long and turned upwards at the end. During the time the animals are sloughing the old hoofs and growing new ones they are very lame and often are in severe pain. Usually they do not move about much and unless feed and water are within easy reach, death may result from starvation and thirst.

As a result of placental transmission of selenium (183) animals may be born with hoofs which show the effects of selenium poisoning (103) (155).

Miller and Williams (99) produced chronic selenium poisoning in horses and mules by feeding small daily doses of sodium selenite over long periods of time.

The symptoms were similar to those of the "alkali disease" but were not quite as pronounced.

In hogs the symptoms of "alkali disease" are emaciation, lameness, loss of hair from the body and irregular growth of the hoofs with occasional sloughing of the hoofs similar to the condition in cattle and horses. When young pigs are fed corn containing 10 p.p.m. to 15 p.p.m. of selenium, the symptoms appear within two or three weeks.

Beath (5) has observed that sheep on seleniferous ranges produce lambs with abnormal eyes and deformed feet. In 1935 Draize and Beath (29) published data on 100 autopsies of cattle and sheep suffering from either "blind staggers" or "alkali disease." This report covers both gross pathology and microscopic pathology. Following is a brief summary of their report.

Pathology of "blind staggers." Atony of the smooth muscles of the gastro-intestinal tract, gall bladder and bladder is common. The blood vessels of the viscera are congested. In winter cases impaction of the rumen is usually observed. A stasis of the food material is evident also in the omasum. The abomasum and upper small intestine show irritation of varying degrees and petechial hemorrhages and ulceration are noted in severe cases. The large intestine is usually free from irritation. Peritoneal blood-vessels are congested and ascites is common.

The liver is acutely congested and areas of focal necrosis appear early in the intoxication. These necrotic areas appear to be connective tissue scars which on contraction form pits upon the surface of the organ. Cirrhosis is not as prevalent in "blind staggers" as it is in "alkali disease". The gall bladder may be enlarged to twice normal size and the walls often exhibit mucoid degeneration and congestion of the blood vessels. There is congestion in the medulla of the kidney and kidney stones are not uncommon in the more advanced cases.

Examination of the heart revealed endocarditis, myocarditis and petechial hemorrhages of the epicardium. Erosion of the long bones, especially the tibia, is found in about two-thirds of the cases. In typical cases of "blind staggers" the hoofs are not involved.

"Alkali disease." The lesions of "alklai disease" are of much more chronic nature than those of "blind staggers." At autopsy the heart and liver show the most severe lesions. In the advanced cases the heart is invariably atrophied (dish-rag) and shows evidence of decompensation. Bundles of muscle fibers appear to be separated by a sero-fibrinous exudate. Petechial hemorrhages on the epicardium are not as common as in "blind staggers" but endocarditis and myocarditis are prevalent.

The liver is cirrhotic and often atrophied. Microscopic examination reveals changes typical of cirrhosis. The kidney is usually severely damaged in the "alkali disease" and glomerulo-nephritis is observed in many cases. Both the medulla and cortex of the adrenals are hemorrhagic. Lesions in the gastro-intestinal tract are of the same general character as observed in "blind staggers" except that the intense irritation is not observed. Erosion of the joints of the long bones is common in "alkali disease."

A high incidence of anemia is found in both "alkali disease" and "blind stag-

gers." The reviewers have observed that the hemoglobin content of the blood can be used to detect early stages of the "alkali disease" in cattle. In severe cases the hemoglobin may drop as low as 7.0 grams per cent (164).

Selenium Poisoning in Poultry. Poor hatchability of eggs has always been a common complaint among farmers in "alkalied" or seleniferous areas and this defect can be used as an aid in locating such areas. Hatchability is influenced by concentrations of selenium in the feeds that are too low to cause manifest symptoms of poisoning in other farm animals. Chicks that do hatch from seleniferous eggs are weak and have a wiry appearing down. Experimental work has shown that the developing chick embryo is extremely sensitive to selenium poisoning.

Before selenium was suspected as the cause of "alkali disease," Franke and Tully (50) obtained eggs from a farm in an "alkalied" area and incubated them at the South Dakota Agricultural Experiment Station. Contrary to the popular belief, nearly all the eggs were fertile. Upon examination only 6 out of 133 eggs showed no signs of a developing embryo. However, only 5 chicks actually hatched, and 2 of these died within 2 days. The rest of the fertile eggs contained deformed embryos in various stages of development. Some of the monsters were alive but were unable to make their way from the shell because of their deformities. In most of the monsters the upper beak was very short or missing, many of the embryos had deformed legs and wings and some lacked eyes.

Tully and Franke (178) then found that a ration containing 65 per cent of grain from an "alkalied" area inhibited growth of chicks, initial egg production of pullets was delayed and total egg production was reduced. Later, after Robinson (146) found selenium in the toxic wheat which Franke had supplied, it was shown (103) that inorganic selenium produced the same effects in chickens as had previously been noted from toxic grains. This work has also shown that chickens appear to be more sensitive to poisoning by inorganic selenium than white rats. Decreased feed intake and production of monsters in the eggs was noted when White Leghorn hens were fed a ration containing 3.5 p.p.m. of selenium as sodium selenite.

In the ration of young chicks 8 p.p.m. of selenium as sodium selenite caused depression in growth, but 4 p.p.m. had no apparent effect.

As further evidence that the deformities of the embryos were caused by selenium Franke et al. (38) injected small amounts of sodium selenite into the air cells of fertile eggs before incubation. Selenium in amounts as low as 0.0005 mgm. per egg (0.1 p.p.m.) caused typical deformed embryos.

When rations containing seleniferous grains (15 p.p.m. selenium in ration) were fed to laying hens for seven days the hatchability of the eggs decreased to zero but returned to normal in seven days after the hens were changed back to selenium-free rations (140). One per cent of elemental sulfur in seleniferous rations failed to affect the toxic action of selenium.

Poley and Moxon (139) studied the tolerance levels of selenium in laying rations. Selenium at a level of 2.5 p.p.m. had no appreciable effect upon hatchability, at a level of 5.0 p.p.m. hatchability was reduced slightly, and at a level of 10.0 p.p.m. hatchability was reduced to zero. It has been recommended

that starting rations for chicks contain less than 5 p.p.m. of selenium (141). The selenium content of meat and eggs from hens which had been fed a seleniferous ration for six weeks was reported by Moxon and Poley (116). When the ration contained more than 2.5 p.p.m. of selenium, the meat and eggs contained concentrations of selenium in excess of the suggested tolerance limit for foods (15) (121).

Landauer (80) studied the effect of chronic selenium intoxication in laying Creeper hens on their progeny. He found that the Creeper mutation exaggerated the interference with normal development caused by selenium. Except for this study by Landauer, all of the published work on selenium poisoning in poultry has been done at the South Dakota Agricultural Experiment Station.

Experimental selenium intoxication in laboratory animals. The experimental investigation of the action of selenium on the animal body has been stimulated by two interests. The earliest interest was in the action of toxic doses of selenium to show the pharmacological relationship of this element to other well known poisons. The second interest is in the effect of relatively small amounts of selenium ingested over a long period of time. It is now recognized that chronic selenium poisoning of animals has been known for nearly a century, but the possibility of widespread chronic selenosis in humans has been recognized only in the past decade.

Acute toxicity of selenium. Czapek and Weil (27) credit Japha (70) with the first investigation of the toxicity of selenium. Japha reported in 1842 on the action of metallic selenium and various selenium salts on men and animals. His subjects were chiefly local animals and himself. Rabuteau (143) in 1869 published the results of his investigations on the properties and elimination of selenium (and tellurium) from the animal body. He contended that characteristic blood changes were produced, and that death was due to mechanical effect of certain crystals formed in the blood.

As the result of work extending over several years, Czapek and Weil (27) reported the pharmacological action of toxic doses of selenium on cold blooded animals (the frog) and warm blooded animals (rabbits, cats and dogs). They used sodium selenite and selenious acid as sources of selenium, assuming that the selenates were toxic to the animal only after reduction to selenite. The principal results and conclusions of Czapek and Weil's work may be summarized as follows:

The lethal dose for the frog is less than 0.1 mgm. of selenium. The essential action is a narcosis of the central nervous system followed by stoppage of the heart in an engorged condition in diastole. In general the action of the sodium selenite on the heart was contrary to that of digitalis, and the two compounds counteract each other to a certain extent. They concluded that sodium selenite acts on the ganglia of the heart or some other regulatory mechanism rather than directly on the heart muscle itself. Death is due to a combination of the effects on the heart and on the central nervous system. There was no difference between the action of sodium selenite and sodium arsenite but the latter seemed to kill frogs more quickly in the same dose.

In warm blooded animals the first symptoms appear about 5 minutes after

subcutaneous injection of selenite, and 15 minutes after oral administration. The breath acquires a garlic odor, and the animal shows signs of nervousness and fear, evidenced by loud crying. This is followed by vomiting, then quietness and somnolence. Respiration becomes difficult, leading to dyspnea. The reflexes decrease in intensity but the heart remains normal up to this time. The dyspnea is followed in turn by opisthotonus, tetanic spasms in the muscles of the extremities (interfering with respiration), clonic spasms and death. A marked symptom is a gradual fall in blood pressure, beginning about $\frac{1}{4}$ to $\frac{1}{2}$ hour after injection (before the respiration and reflexes are affected) and continuing to death. It is concluded that the primary action of the selenium is on the central nervous system and death is due to respiratory failure. The effect on blood pressure is due to action on the splanchnic nerve system.

Some hemorrhagic and destructive changes were noted in the intestines. These were more severe in carnivora than herbivora. Greater amounts of selenate salts are required to cause the same effect as selenite, and the action is somewhat slower. They observed no consistent changes in the blood picture.

It is regrettable that Czapek and Weil did not describe their experiments more completely. However, Modica (101) confirmed the results of Czapek and Weil and observed further that in animals chronically poisoned by selenious acid, wasting is always a marked feature. Also, tolerance to small doses was very difficult to establish in the case of selenium. He pointed out that these two symptoms distinguished the action of selenium from that of arsenic. Crompton (26) further studied the action of selenium (and tellurium and arsenic) on isolated frog tissues and confirmed the observations of Czapek and Weil. He concluded that neither selenite nor selenate had any direct poisoning action on the frog heart, gastrocnemius muscle or isolated motor nerve trunks. Also, there was no action of these salts directly on the blood vessels. The selenium acts on the central nervous system.

In more recent work of the same nature Anderson and Moxon (3) have confirmed nearly all the symptoms described by Czapek and Weil for acute selenium poisoning in the dog.

As Painter (129) has pointed out, there is considerable disagreement about the minimum lethal or minimum fatal dose of selenium for various animals. The different results reported are due to species differences in susceptibility, mode of administration, and possibly to impurities in compounds used (3) (98) (120) (129) (159) (195). Expressed in milligrams of selenium per kilogram of body weight, the following values are representative of the least amount of selenium that is fatal. By subcutaneous, intraperitoneal or intravenous injection the fatal dose of sodium selenite is from 3.0 to 5.7 for the rat, 0.9 to 1.5 for the rabbit, and about 2.0 for the dog. In general sodium selenate is less toxic than the selenite, the amount needed to kill varying from 3.0 to 5.9 for the rat, and 2.0 to 2.5 for the rabbit. For larger animals Miller and Williams (98) state the lethal dose of sodium selenite is <4.4 for the horse, 3.3 for the mule, 11.0 for the cow, and 15.0 for the pig. Moxon (106) found the toxicity of selenium in *d, l*, seleno-cystine to be intermediate between that of selenite and selenate,

namely 4.0 mgm. per kgm. All reports agree that selenium metal is non-toxic because of its insolubility.

In determining the relative toxicity of arsenic, selenium, tellurium and vanadium, Franke and Moxon (39) administered the sodium salts of all the elements by intraperitoneal injection. The relative toxicity in decreasing order was: tellurite, selenite, vanadate, arsenite, selenate, arsenate and tellurate. No deaths were noted from 160.0 mgm./kgm. of molybdenum as ammonium molybdate.

Smith, Stohlman and Lillie (159) noted that repeated small doses of selenium were cumulative in effect, although the amount required to kill in this manner was greater than in a single dose. They found no evidence of a tolerance being acquired, as had been reported by Modica (101). Marked individual variation in susceptibility to selenium toxicity was noted for rats, cats, and rabbits, and the same has been reported for dogs (3).

The characteristic blood changes which Rabuteau (143) ascribed to selenium poisoning have not been observed by subsequent investigators (26) (27) (195). The only recent study of the changes in the blood picture due to acute selenium poisoning is that of Anderson and Moxon (3). They summarized the characteristic blood changes in dogs as follows: Marked rise in hemoglobin and as much as 62 per cent rise in hematocrit. Decrease in inorganic phosphorus, nonprotein nitrogen, calcium, ascorbic acid, and blood sugar. Reduction in glutathione content of the blood was indicated. No effort was made to determine the presence of the characteristic crystals in the blood described by Rabuteau, but not found by other workers.

Recently Moxon and Rhian (117) have investigated the effects of massive doses of selenium as sodium selenite (20 mgm./kgm.) administered subcutaneously to dogs. It has been observed that death results in 25 to 30 minutes after administration of the selenium. This observation may be used for comparison with other investigators' reports that doses of 4 mgm./kgm. or more of selenium result in death "in a few minutes," or "in a relatively short time."

That the toxicity of sodium selenite injected intraperitoneally can be influenced by other compounds was reported by DuBois, Rhian and Moxon (31). Reduced glutathione, in a ratio of 10 moles to one mole of selenium, when injected two hours before the selenium prevented death in 8 of 10 rats given 3.0 to 3.5 mgm. of selenium per kilogram. The same amount of glutathione was ineffective when 4.0 mgm./kgm. of selenium was given, showing that large excesses of GSH are necessary for the protective effect.

Dudley (34) has reported that selenium oxychloride is extremely vesicant and toxic. When 0.01 cc. was applied to the skin of a rabbit death occurred in less than 24 hours. The reviewers have noted that one drop of the compound applied to the skin of a dog produced death in a few hours.

Sub-acute and chronic toxicity of selenium. The comprehensive investigation of the chronic toxicity of selenium was begun by Franke in 1928 as stated earlier in this review. Since then many investigators have shown that symptoms produced by feeding naturally toxic (seleniferous) rations and normal

(selenium-free) rations with added inorganic selenium are virtually identical (40) (44) (46) (57) (94) (121) (150). The more important symptoms of sub-acute selenium poisoning in the white rat described by Franke (36) and Franke and Potter (45) may be summarized as follows: *a*, marked restriction of food intake; *b*, decreased growth; *c*, marked, progressive anemia, and *d*, definite pathological changes especially in the liver. Rats fed a diet with a selenium content which produces a condition of sub-acute toxicity restrict their food consumption to as little as 25 per cent of that of rats fed a similar diet without selenium. Most of the rats assume a characteristic hunched posture. In a few cases the hind legs become paralyzed. The fur becomes rough and is stained a dark yellow around the genitals. In rats that die in a short time, gross pathology is not evident. The most marked symptom is dilatation of the veins of the visceral region. The lungs and liver usually have a congested appearance. The reproductive organs are undeveloped and may show degeneration. The intestines show evidence of hemorrhage; the animals are jaundiced and very emaciated.

Rats fed a ration with a lower selenium content may be said to show symptoms of chronic poisoning. They may show slight increases in weight and appear to be in fair condition, but their reproduction may be impaired (48). In many cases a definite breakdown of vital organs takes place. They develop an anemia of progressive severity and die with hemoglobin levels as low as 2.0 grams per 100 cc. blood. The most outstanding pathology is in the liver which is atrophied, necrotic, cirrhotic and hemorrhagic in varying degrees. In cases of active liver regeneration it is usually found that the right lateral and/or the caudate lobes are hypertrophied. The left lateral and central lobes are most susceptible to atrophy. In many cases the heart and the spleen are enlarged. Ascites and pleural edema are common. In some instances death results from internal hemorrhage from the liver. Lillie and Smith (91) have studied the histogenesis of the liver changes associated with chronic selenosis.

Recognizing that there are individual variations it may be said that in general symptoms of chronic poisoning in rats are common when the ration contains 5 to 15 p.p.m. of selenium, and sub-acute symptoms are produced when the ration contains 15 to 25 p.p.m. selenium or more.

The tendency for range animals to avoid certain plants, or the common vegetation of well-defined areas, has long been recognized in relation to "alkali disease." An analogous situation has been shown to exist in laboratory feeding trials. Rats given a choice of rations invariably avoided those containing either naturally occurring selenium or sodium selenite (47). Miller and Schoening (100) noted that young pigs were loath to eat corn containing selenium. Admittedly, the lower food intake of the animals accounts for some of the symptoms of selenium toxicity, but Franke and Potter (45), Franke and Painter (42) and Munsell, DeVaney and Kennedy (121) have shown that the other symptoms and at least part of the growth effect are due to the selenium in the ration.

Relatively little experimental work on chronic selenium poisoning has been done with laboratory animals other than the rat. Smith, Westfall and Stohl-

man (161) (162), Smith, Stohlman and Lillie (159), and Westfall, Stohlman and Smith (183) have reported investigations using cats and rabbits. Moxon (107) studied selenium poisoning in pigs under laboratory conditions and Rhian and Moxon (144) have used 34 dogs in studies on the toxicity of orally ingested selenium. Ellis et al. (35) have studied selenium poisoning in fishes which were kept in water containing small amounts of selenium.

Smith and his co-workers have been especially interested in securing information on the chronic toxicity of selenium that may be directly applicable to interpretation of their survey data on selenium poisoning in humans. They noted in some of their work (161) that the susceptibility of the cat, rabbit and rat to selenium poisoning decreased in that order. The cat, in common with other carnivora, showed more intestinal symptoms than the rabbit. It is also interesting that these investigators have found no anemia in cats suffering from chronic selenium poisoning. In later reports (156) (157) they concluded that the species differences in susceptibility could be accounted for on the basis of the selenium : protein ratio of the diet. It is still questionable whether this conclusion can be applied to species differences mentioned in other reports.

In their work with dogs, Rhian and Moxon (144) found that these animals exhibited most of the symptoms shown by the rat, and many shown by larger (farm) animals. In general the dog is more susceptible to chronic selenium poisoning than the rat.

The principal symptoms of chronic or sub-acute selenium poisoning described for the dog are as follows:

As shown by voluntary restriction of food intake and sub-normal growth, as little as 7.2 p.p.m. of selenium in the ration in the natural form (in corn) and 10 p.p.m. as added sodium selenite were toxic. As added sodium selenite, 20 p.p.m. selenium in the ration induced almost complete refusal of food and death in a very short time. This amount of selenium in the natural form produced severe nervous disorders (resembling "blind staggers"). Pathologically the liver and spleen were most severely affected. Small localized hemorrhages were noted in the intestines. Severe ascites was quite common. The dogs became markedly emaciated and the hair became coarse and rough. In the later stages of intoxication apathy was common.

Chronic or sub-acute selenium poisoning has little effect on the commonly determined blood constituents of the dog, except hemoglobin. Anemia in dogs becomes progressively more severe as the selenium poisoning progresses, terminal values for hemoglobin being as low as 3.5 grams per cent. Phosphatase activity of the blood is increased and non-protein nitrogen is slightly reduced.

Distribution of selenium in the body. The occurrence of selenium in the animal body has been shown to be related to the amount and form of selenium administered, and length of time of administration. Dudley (32) has studied the distribution of selenium in acute cases of poisoning and Smith, Westfall and Stohlman (161) have summarized the limited information available up to 1937, on the distribution of selenium in the animal body.

In cases of acute selenium toxicity, there is excretion of selenium as a volatile,

very odoriferous compound in the breath. It has been claimed that this compound is methyl selenide (61), but definite proof of this is lacking (151). Miller and Williams (98) obtained some information on the amount of selenium retained by horses, mules, pigs, and a calf in cases of acute poisoning. In general they found selenium in all tissues examined except hide and hair. The kidney, liver, lung and spleen invariably contained the most selenium, and its occurrence in less active tissues of the body was apparently in direct ratio to the time of survival.

Smith, Westfall and Stohlman (162) studied the distribution of selenium in doses up to two-thirds of the minimum lethal dose. It was noted that as soon as 5 minutes after the intravenous injection of sodium selenite the concentration in the kidney exceeded that in the blood. The selenium content of the kidney and liver increased, and that of the blood decreased for 6 hours after which there was a continued gradual excretion of the selenium from all parts of the body. It was noted that in all cases the concentration of selenium in the red blood cells was greater than that in the blood plasma.

Through the use of radioactive selenium McConnell (95) has studied the distribution of a single sub-toxic dose of sodium selenate in the body of the rat up to 96 hours after administration. A wide but varied distribution of selenium was found in the tissues examined. The greatest concentration of selenium was found in the liver, muscle, gastro-intestinal tract and blood. Lesser amounts of selenium were found in lung, spleen, heart and tumor and none was found after 24 hours in skin, fur, teeth or long bones. Small amounts were deposited in tumor tissue. Selenium was excreted chiefly through the kidney, only a small amount being excreted in the feces. In a later report (96) it was noted that 3 to 10 per cent of a single dose of selenium was exhaled in the first 24 hours. The volatile compound was not identified.

The distribution of selenium in the animal body after chronic poisoning has been studied thoroughly by Dudley (32), Smith, Westfall and Stohlman (161), Munsell, DeVaney and Kennedy (121), Moxon (103), Rhian and Moxon (144) and Anderson and Moxon (1).

After oral administration of selenium to produce chronic poisoning, all the tissues of the body contain some selenium. To some extent, the amount and kind of selenium in the ration and the length of the period of administration influence the amounts in various tissues. Munsell, DeVaney and Kennedy (121) found that when rats were fed a seleniferous ration over a long period of time, the selenium content increased to a maximum during the first 4 to 8 weeks of feeding and remained constant thereafter. If the poisoning did not become increasingly severe, the excretion balanced the intake. Excretion takes place through three channels, the breath, urine and feces. Smith, Westfall and Stohlman (161) have shown that in the cat 50 to 80 per cent of the total selenium is excreted in the urine and from a trace up to 18 per cent in the feces.

In the body the highest concentration of selenium is found in the liver, kidneys, spleen, pancreas, and lung. Other less active tissues contain relatively much less selenium. It was noted (162) that more selenium is retained in the

body when the ration contains naturally occurring organic selenium than when it contains sodium selenite. Conversely, urinary excretion of sodium selenite is greater. Using a ration containing 18 p.p.m. selenium, Anderson and Moxon (1) using smaller concentrations of selenium than used by Smith, Westfall and Stohlman, noted no difference in retention of naturally occurring selenium and sodium selenite. Retention of selenium was a maximum during the first week of feeding, and steadily decreased after that time. When the rats were placed on a selenium-free ration, most of the selenium was excreted from the body within two weeks after the change. Individual variation in selenium content of the tissues is very marked (144).

Data obtained by the reviewers and others (164) indicate that when cattle graze on naturally seleniferous plants, the distribution of selenium in the body is qualitatively the same as that noted for experimental animals. For eight steers that had been on seleniferous range for two seasons the average analysis of body tissues (on the fresh weight) showed a concentration of 3.05 p.p.m. selenium in the hoof, 2.2 p.p.m. in the kidney and 1.2 p.p.m. in the liver. All the other parts of the carcass were lower in selenium content, the muscle containing 0.88 p.p.m. For 30 steers that had been on the same range for three seasons the average selenium content of the liver was 5.7 p.p.m., the kidney 4.1 p.p.m., and the muscle 3.0 p.p.m.

The characteristic pathology of the stomach noted in chronic selenium poisoning has aroused interest in its effect on the function of this organ. Smith and Stohlman (158) have reviewed the meager literature on this subject up to 1940. In their own experiments they found that chronic selenium poisoning in rats and cats had no essential effect on free or total gastric acidity.

From a study of the effects of chronic selenium poisoning on liver function in rabbits and cats, Smith, Westfall and Stohlman (163) concluded that liver function measured by the rose bengal, bilirubin and hippuric acid tests could be correlated with the structural changes in the liver. The rose bengal test was most sensitive in measuring liver dysfunction due to selenosis.

A number of investigators have commented on the effect of selenium on blood sugar and liver glycogen. Wright summarized the information available to 1941 (194). From his own work he concluded that doses of sodium selenite greater than 5.0 mgm./kgm. cause increase in blood sugar and over 10.0 mgm. cause decrease in liver glycogen in the rat. In the rabbit, 3.0 mgm./kgm. cause an increase in blood sugar, followed in the fasted rabbit by a sharp fall preceding death. He also noted that fasted rats injected with sodium selenite have a lowered glucose tolerance and delayed deposition of liver glycogen from the injected glucose. The observations of Svirbely (170) and Lardy and Moxon (82) suggest that ascorbic acid may play an important rôle in the detoxification of selenium.

Effect of selenium on the blood. Franke and Potter (45) observed that one of the symptoms of chronic selenium poisoning in rats was a progressive anemia. Terminal values as low as 2.0 grams per cent hemoglobin were noted. This effect was first noted when the ration contained naturally toxic grain and has

been shown to be caused also by rations containing sodium selenite (46) (150). Smith, Stohlman and Lillie (159) have further studied the anemia of the rat and observed that the chief characteristics are "relatively low hemoglobin compared to the red count, marked reticulocytosis which at times may be as high as 80 per cent, anisocytosis, polychromatophilia and normoblasts in greater or lesser numbers."—"The anemia when well developed is essentially microcytic and hypochromic." These same workers observed that cats seem to be less inclined to show anemia in chronic selenium poisoning. Only two out of fourteen cats given daily doses of 0.2 to 0.25 mgm. selenium per kilogram showed definite anemia.

Progressive anemia is a constant symptom of selenium poisoning in dogs (144). Hemoglobin values of 4.0 grams per cent were noted in dogs that lived a relatively long time on a ration containing selenium. In other instances, terminal values of 3.5 grams per cent have been observed in young puppies severely affected by chronic selenium poisoning (117).

Aside from the characteristic anemia, chronic selenium poisoning of the dog seems to have no characteristic effect on the blood picture (144). Preliminary data indicate an elevation of blood phosphatase values, and a slight reduction in non-protein nitrogen. (This effect on phosphatase is contrary to that observed by Bauer (4).)

Observations on the blood picture of cattle grazing on seleniferous range have failed to reveal any pronounced changes except for the hemoglobin levels (164).

The effect of dietary factors on selenium poisoning. Early in the investigation of the "alkali disease," Franke and co-workers (103) attempted to find a dietary factor (or factors) that would prevent or reduce the toxicity of seleniferous rations. Various feeding trials with rats showed that changing the calcium and phosphorus content (2.8 per cent to 11.2 per cent tri-calcium phosphate) and the Ca:P ratio of the ration (1:2 to 1:6) had no beneficial effect. That vitamins A and D had no close relationship to selenium poisoning was shown by feeding a ration containing up to 4 per cent of cod liver oil. Yeast as a source of vitamin B complex (up to 0.8 gram per rat per day as a supplement) had no beneficial effect. The combined effect of supplements of orange juice, dry yeast and cod liver oil gave improved growth to rats but did not change the characteristic pathology due to selenium. In more recent work (164) it has been noted that daily administration of crystalline vitamin B_1 (either orally or by injection) to rats fed a ration containing 12 p.p.m. of selenium greatly increased the severity of the symptoms.

Cystine to the extent of 5 per cent of the ration had no alleviating effects on selenium poisoning and at the higher level seemed to be slightly toxic itself. Schneider (150) also fed cystine at concentrations of 0.2, 0.4, and 0.6 per cent of the ration and noted no beneficial results. Sulfur fed at 0.5 per cent of the ration was not beneficial in selenium poisoning and itself was slightly detrimental to rats and chickens (103).

The effect of the protein content of the ration on selenium poisoning has been investigated (56) (90) (103) (153) (157). From feeding rations of high, normal,

and low protein content (with equal calorific value) it was concluded by the South Dakota workers (103) that selenium poisoning was less severe on the ration with a high protein content. This conclusion has been substantiated by Smith (153) and Smith and Stohlman (157) who have extended the observation as a possible explanation for certain previously observed species differences in susceptibility to selenium poisoning. Expressed on the basis of per cent protein in the ration and micrograms of selenium per 100 grams of ration, they concluded that a ration with a protein : selenium ratio of 1:30 or less is non-toxic while a ration with the same selenium content and a protein : selenium ratio of 1:100 is dangerously toxic. Contrary to the finding of Smith and Stohlman (157) that neither cystine nor methionine were themselves of value in alleviating the toxicity of selenium, Schultz and Lewis (151) found that addition of methionine to a methionine deficient ration did have beneficial effects. Probably the protein effect noted is due to more than the methionine content of the diet (157). The proteins which, it is claimed, have definite value in the alleviation or prevention of chronic selenium poisoning are: casein, lactalbumin, ovalbumin, gelatin, proteins derived from wheat, dried brewers' yeast, desiccated liver, and zein. The workers mentioned above do not agree on the action of all these proteins.

Of further interest is the observation reported by Moxon (108) that of commercial proteins fed to rats crude casein and linseed meal were the only ones that prevented the characteristic liver lesions. Crude casein gave excellent growth response and linseed meal gave the best growth of the vegetable proteins used, but not as good as casein or dried liver. Dried whole beef liver, Labco (purified) casein, and whole milk powder all gave good growth, but failed to prevent or alleviate pathological lesions of selenium poisoning. Meat scraps, tankage and corn gluten meal gave poor growth and no protection against selenium poisoning.

In experiments with dogs it was noted that dried beef liver (18 per cent of the ration) gave excellent growth and prevented all the symptoms of selenium poisoning on a ration containing 10 p.p.m. selenium. Crude casein and tankage gave poor growth and no protection against selenium poisoning. Linseed meal gave good protection in dogs, and was the only protein to protect both rats and dogs.

The species differences shown by this work with rats and dogs emphasizes the need for experimental work with farm animals before any practical applications can be recommended.

It may be well to mention that although Smith and Stohlman (157) found good protective action by the proteins of wheat and corn for the rat, experiments with dogs in this laboratory have shown no particular protective action for these proteins (164). In fact, the most severe cases of poisoning in dogs have occurred on rations containing large amounts of these proteins.

There is disagreement concerning the effect of the fat content of the ration on selenium poisoning. Moxon (103) reported that in a ration containing 37.5 p.p.m. of selenium the percentage of fat in the ration had very little influence on the toxicity of selenium. Smith (153) observed some beneficial effect from a ration of low protein and high fat content, but suggests the result may come from the protein sparing action of the fat.

Effect of other elements. In experiments designed to show the effect of certain other elements administered concurrently with selenium (104) (110) (164) it has been noted that fluorine, molybdenum, chromium, vanadium, cadmium, zinc, cobalt, nickel and uranium caused an increase in mortality. All these elements were given in the drinking water at a concentration of 5 p.p.m. with a ration containing 11 p.p.m. selenium. The same amount of soluble tungsten prevented, to a limited extent, the typical liver lesions caused by selenium and decreased mortality. A level of 2.5 p.p.m. tungsten reduced mortality slightly but did not prevent liver lesions.

Surprisingly enough, 5 p.p.m. of arsenic as sodium arsenite administered in the same manner completely prevented the symptoms of selenium poisoning. Greater feed consumption and better growth resulted from giving 2.5 p.p.m. of arsenic, but liver lesions were not entirely prevented. DuBois, Moxon and Olson (30) have further shown that sodium arsenite and arsenate are equally effective in counteracting the effect of selenium in seleniferous wheat, while arsenic sulfides (AsS_2 and AsS_3) are ineffective. Sodium arsenite was equally effective against selenium in the form of seleniferous wheat, sodium selenite and the selenium analogue of cystine. It was noted that 10 p.p.m. arsenic in the ration gave slightly better results than 5 p.p.m. in the drinking water, due to a slightly higher arsenic intake. Arsenic was effective in treating rats that had been fed the seleniferous ration for 20 days previous to beginning the arsenic treatment, but was ineffective after the rats had been on the toxic ration for 30 days.

Lead had no effect on the toxicity of selenium, but bismuth (subnitrate) had a slightly beneficial effect.

It has been noted that growing chicks are more sensitive to arsenic than rats or dogs, and the arsenic treatment of chicks is thus less successful than the treatment of rats or dogs (108).

Subsequent work with chickens (108) has shown that 10 p.p.m. of arsenic in the drinking water nearly counteracts the effect of 14 p.p.m. of selenium in the ration. More recent studies (164) have shown that arsenic markedly improves hatchability of eggs from hens fed a seleniferous ration.

Moxon (107) has reported prevention by arsenic of selenium poisoning in a pig raised under laboratory conditions, and Rhian and Moxon (144) have shown that 5 p.p.m. of arsenic in the drinking water will prevent selenium poisoning in dogs fed a ration containing approximately 10 p.p.m. selenium.

Recent work in the reviewers' laboratory (164) has further substantiated the relationship of the time of administration of selenium and arsenic noted by DuBois, Moxon, and Olson (30). When an amount of selenium equivalent to that consumed in a ration containing 12 p.p.m. selenium was injected daily and 5 p.p.m. of arsenic in the water was given ad. lib., death resulted in over half the animals. When the selenium was given in the ration and arsenic equivalent of that consumed in the water was injected daily, no deaths resulted. When the selenium was in the ration and arsenic in the water, no deaths resulted.

Arsenic in the form of certain organic compounds (neoarsphenamine and sulfarsphenamine) equivalent to 5 p.p.m. arsenic in the drinking water, was not quite as effective as sodium arsenite at the same level of arsenic.

It has been noted that antimony trichloride fed in the ration has about the same activity as organic arsenic in preventing selenium poisoning. Sodium antimoniate has given no protection against selenium poisoning.

Effect of certain organic compounds. Stekol (166) showed that bromobenzene and naphthalene were detoxified in the body of the rat, dog and rabbit by conjugation with tissue cystine and methionine and excreted in the urine as *p*-bromophenyl-mercapturic acid. The close relationship between sulfur and selenium compounds suggested an investigation of the action of bromobenzene and similar compounds in selenium poisoning. Moxon et al. (118) reported that administration of bromobenzene to rats and dogs fed a seleniferous ration increased the urinary selenium output. Moxon (108) noted that administration of either bromobenzene, benzene or naphthalene to steers grazing on seleniferous range caused a rise in urinary selenium output. The selenium content of the blood decreased in most cases. This observation suggests that these aromatic compounds aid in converting tissue selenium into a compound that is readily excreted from the kidney.

These observations suggest that much more work should be done on the nature of compounds that will promote excretion of selenium from the animal body.

Effect of selenium on enzyme systems. That selenium has direct effect on certain unicellular organisms and enzyme systems has been known for many years. Czapek and Weil (27) state that Chabrie (22) had found sodium selenite to inhibit alcoholic fermentation. They concluded that the growing yeast cells reduced the selenite to metallic selenium. This inhibiting action was later confirmed by Woodruff and Gies (195) and more recently by Moxon and Franke (112) who extended the observation to show the relative toxicity of selenium and other metals to fermentation systems. They found that during yeast fermentation of glucose, as measured by CO_2 production, selenium was more toxic than vanadium, arsenic, and tellurium. The toxicity of sodium selenite, selenide and selenate decreased in that order. Sodium sulfide partially counteracted the toxic effects of the selenium salts, but elemental sulfur had almost no action of this type. Sodium sulfite, ammonium sulfate and sodium thiosulfate were unable to counteract the toxic effects of selenium. Later Lardy and Moxon (81) noted that 0.2 mgm. of arsenic would almost completely counteract the effect of 1 mgm. of selenium in inhibiting the fermentation of glucose by bakers' yeast.

Gastler (53) found that selenium salts had no effect on the saccharogenic activity of malt diastase, taka diastase and amylopsin.

Levine (88) has reviewed the literature pertaining to the action of selenium salts on bacterial growth and the action of such cultures in reducing selenium compounds.

The action of selenium salts on isolated enzyme systems has been investigated

for various purposes. Collett (23) showed that selenite and selenate inhibit the action of succinoxidase, and in later work Collett and co-workers (24) (25) have used selenium to determine the specificity of certain dehydrogenases.

Labes and Krebs (76) found that 0.02 per cent sodium selenite inhibited the action of succinoxidase of pig's muscle. Potter and Elvehjem (142) demonstrated that by using sugars as substrates, selenium caused 80 per cent inhibition in the oxygen uptake of living yeast cells. Less than 10 per cent inhibition was noted when pyruvate or lactate was used as substrate. These workers suggest the action of the selenite may be indirect, through its action on glutathione as has been suggested by Bersin (13).

Wright (191) showed that selenium inhibits the oxygen consumption of mammalian (rat) tissue slices. Using glucose or succinic, pyruvic, lactic or citric acids as substrate, kidney, brain, muscle and tumor slices were incapable of oxidation. With the liver slices the action of selenium was an initial stimulation followed by inhibition. All the tissues rapidly oxidize p-phenylene-diamine in the presence of selenium. Reduced glutathione or pyruvic acid would protect the tissue from the action of selenium if they were added with the selenium. If the selenium was allowed to act for a time before the glutathione or pyruvic acid was added, the oxygen consumption was not restored. Wright concluded that selenium is a general hydrogenase inhibitor. Later Wright (192) measured the effect of diselenodiacetic acid on tissue slices. The selenium in this compound inhibited the oxygen consumption of slices of rat liver, brain, and kidney, but had no effect on muscle tissue. Anaerobic glycolysis of liver, kidney, or tumor slices was not affected by this organic compound.

While investigating the effect of selenium on rat livers Wright (193) found that selenium as sodium selenite, selenate or diselenodiacetic acid inhibits the activity of urease but has no specific effect on liver arginase. This further indicates a relationship between selenium and those enzymes dependent on the action of sulfhydryl groups.

Bernheim and Klein (12) have classified enzymes on the basis of their reaction to or with selenium into three groups: "those relatively insusceptible to selenium, including the glucose, lactate, and pyruvate oxidases of brain and probably l-tyrosine, xanthine, and alcohol oxidases of liver; (b) those in which selenium catalyzes the destruction of an active group, including succinoxidases, choline oxidase, d-proline oxidase, and tyramine oxidase; and (c) l-proline oxidase which is inactivated immediately, suggesting that selenium combines with an active group".

It seems that work on the specific action of selenium on the enzyme systems of the animal body is in its infancy, and much more information is required before the exact mechanism of selenium poisoning can be elucidated.

Organic selenium compounds. Painter has reviewed the literature on organic selenium compounds up to 1941 (129) so only those compounds of immediate biological interest will be discussed here.

The early work of Franke (37) and Franke and Painter (41) (42) (131) (133) (130) showed definitely that the selenium in toxic cereals was associated with the

proteins of the cereals. The studies indicated that the selenium is an integral part of the protein molecule and consequently there has been much speculation regarding the possibility of selenium replacing sulfur in cystine and methionine. The selenium analogue of cystine has been prepared by Fredga (51) (52) and Painter (135). Painter (135) has also recently prepared phenyl-seleno-cysteine, benzyl-seleno-cysteine, benzyl-seleno-homocystine.

Painter, Franke and Gortner (134) synthesized organic diselenides, selenium ethers and seleninic acids of acetic, β propionic, n-propyl, and benzyl radicals. They studied the relationship of these compounds and the selenium compounds in plants (133) in regard to their decomposition in alkaline solutions. Moxon, Anderson and Painter (109) have compared the toxicities of these organic compounds and selenium in wheat.

The toxicity of the selenium analogue of cystine (106) (111) is comparable to the toxicity of selenium in seleniferous grains and in sodium selenite, while toxicity of selenium in all other organic selenium compounds investigated has been much less (106) (109) (111). This is further evidence that selenium may occur in cereal proteins as the selenium analogue of a sulfur amino acid. Horn and Jones (62) have isolated a crystalline amino acid complex containing selenium and sulfur from highly seleniferous *Astragalus pectinatus*. They have tentatively assigned a structure to the components of their complex and they state: "It is believed that the selenium in toxic wheat and other grains is combined in the protein as an amino acid having a similar structure."

They have not published data on the toxicity of the amino acid complex but Horn, Jones, and Ringle (63) have published articles on the isolation of a new sulfur-containing amino acid (lanthionine) from sodium carbonate-treated wool. Lanthionine is similar in structure to the selenium containing amino acid complex. Du Vigneaud and Brown (179) have synthesized lanthionine so it should be possible to synthesize the selenium analogue and determine its toxicity.

There is very little definite information on the mechanism of selenium toxicity. There is however, some information on its effect on unicellular organisms and enzyme systems (see section on Effect of Selenium on Enzymes).

Selenite will oxidize sulfhydryl compounds forming disulfide and an unstable RS-Se-SR compound. According to Painter (129) the action of selenite on sulfhydryls may take three courses:

$$\begin{array}{ll} & \overset{\overset{O}{\|}}{} \\ \text{(a)} & 2\,RSH + SeO_2 \rightarrow RS\text{-}Se\text{-}SR + H_2O \\ \text{(b)} & 4\,RSH + SeO_2 \rightarrow RS\text{-}Se\text{-}SR + RSSR + 2H_2O \\ & \qquad\qquad\quad \downarrow \\ \text{(c)} & 4\,RSH + SeO_2 \rightarrow 2RSSR + Se + 2H_2O \end{array}$$

Stekol (167) has reported the preparation of selenium tetracysteine from sodium selenite and cysteine hydrochloride. Using the same compounds Painter (129) reported the preparation of selenium dicysteine as shown below.

$$\begin{array}{l} HOOC\text{—}CH\text{—}CH_2S\text{—}Se\text{-}SCH_2\text{-}CHCOOH \\ \qquad NH_2 \qquad\qquad\qquad NH_2 \end{array}$$

Compounds of the type RS—Se—SR are unstable as shown by reactions (b) and (c) above and are, therefore, difficult to isolate in the pure form. Painter (135) is of the opinion that the reaction of selenite and cysteine would take place according to reactions (b) and (c) rather than by the reaction which Stekol has reported.

Bersin (13) prepared $HOOCCH_2S—Se—SCH_2COOH$ from thioglycolic acid and selenite. He believes that a similar unstable compound is formed from selenite and glutathione. Crude glutathione isolated from the blood of selenized cattle contains some selenium (2). DuBois, Rhian and Moxon (31) have shown that injections of reduced glutathione will protect rats against doses of selenite which ordinarily cause death. The administration of arsenic (either oral or injected) will likewise protect rats against the toxic action of selenium (either oral or injected). This action of arsenic would suggest the possibility of some reaction between arsenic and selenium compounds *in vivo*. Arsenious acid will react with cysteine to form arsenious tri-cysteine (71) (167). Possibly arsenic reacts with selenium compounds in such a way as to keep the selenium compounds from attacking the sulfhydryl compounds of the body.

Public Health. The relationship of selenium poisoning to public health was reviewed by Manville (93) in 1938 and by Smith in 1940 (154).

Smith, Franke and Westfall (155) in 1935 made a survey involving 111 families living in seleniferous areas of Wyoming, South Dakota, and Nebraska to determine the possibility of selenium intoxication through the ingestion of seleniferous foodstuffs. They found that urines from 92 per cent of the individuals examined contained selenium in amounts varying from 2 to 133 micrograms per 100 cc. This, of course, gave definite proof that selenium was being ingested, but they were unable to discover any symptoms pathognomonic of selenium poisoning. Smith and Westfall (160) made field studies in 1936 and were unable to reach any more definite conclusions about the symptomatology of selenium poisoning in humans.

Lemley (85) and Lemley and Merryman (86) became interested in selenium poisoning in humans in connection with several cases of acute dermatitis. Urine specimens from these cases contained selenium in amounts comparable to the selenium content of urines collected from farm animals suffering from selenium poisoning. Lemley and Merryman (85) (86) administered bromobenzene to the patients with dermatitis and found that it gave immediate relief, and most cases were cleared completely within two or three weeks. Westfall and Smith (184) were unable to increase the rate of elimination of selenium from rabbits by the administration of bromobenzene and have discouraged its use in human cases of selenium poisoning. Nevertheless, it is still being used by physicians in seleniferous areas who report that it is the only treatment which will clear up the particular dermatitis which appears to be associated with selenium.

More field and clinical investigations need to be carried out in the seleniferous areas in an effort to establish the symptomatology of selenium poisoning in humans and to arrive at some definite tolerance levels for selenium in foodstuffs.

Dudley (33) has discussed the hazard of selenium poisoning among workers in

industrial operations where selenium is handled. Both Dudley (33) and Hamilton (59) have described the symptoms of human cases of selenium poisoning, caused by the inhalation of volatile selenium compounds. Halter (58) reported a human case of industrial selenium poisoning. Painter (129) has described his experiences in connection with the accidental inhalation of concentrated hydrogen selenide.

Motley, Ellis and Ellis (102) reported experiencing sore throats after working with sodium selenite and after handling animals which had been injected with sodium selenite. The reviewers, however, have never experienced sore throats at any time which could be attributed to the handling of selenium compounds or handling of animals which had been injected with selenium compounds.

Byers (16) discovered some cases of selenium poisoning in humans in Mexico which resulted from soils being contaminated by selenium in the wastes from silver and gold mines.

Sterner and Lidfeldt (168) have determined the selenium content of urine samples from what they call normal subjects. They find more selenium than should be present unless the subjects are exposed to some source of selenium. Selenium has been investigated at different times as a possible cure for cancer. A few typical references on this work are: (28) (72) (149) (181) (182).

Methods of analysis for selenium. Painter (129) has reviewed the methods of analysis for selenium. Most of the selenium determinations which have been made on rocks, soils, and plant and animal tissues have been made by the methods of Robinson et al. (148) and Beath, Eppson and Gilbert (8). During the past two years the reviewers have used the A. O. A. C. method as described by Klein (73). This method is especially accurate for small quantities of selenium.

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BIOCHEMICAL PROBLEMS OF THE CHEMO-AUTOTROPHIC BACTERIA

C. B. VAN NIEL

Hopkins Marine Station of Stanford University, Pacific Grove, California

1. *Development of the concept of chemo-autotrophism.* When Winogradsky came to de Bary's laboratory, he undertook to study the then vital problem of the alleged pleomorphism of the "sulfur bacteria." For an exact investigation pure cultures were desirable, if not imperative, and it was undoubtedly anticipated that with such cultures it would be an easy matter to conquer the last important stronghold from which the doctrine of bacterial pleomorphism could still be defended. But the early attempts at growing the organisms in laboratory media led to developments in the field of physiology whose significance overshadows the final demonstration that the putative morphological variability exists no more in this group than among other bacterial species. Careful observations and deductions, supported by a few simple, conclusive experiments, led the young scientist to the revolutionary concept that certain micro-organisms, although devoid of chlorophyll, can nevertheless maintain themselves, and even develop, in the absence of organic substances.

Typical for the natural habitat of the sulfur bacteria is the invariable presence of hydrogen sulfide; the organisms themselves are found stuffed with sulfur globules. The correlation had given rise to interpretations involving a causal relationship, it being held that the sulfide was produced from sulfur by the sulfur bacteria. Winogradsky's earliest experiments, however, showed convincingly that this could not be so. And, struck by the gradual disappearance of the sulfur globules from cells which were kept for some days in various culture solutions, he supplied additional hydrogen sulfide to the cultures, whereupon sulfur droplets soon reappeared in the organisms. From these observations Winogradsky conceived the causal relation to be otherwise. The bacteria would be restricted to a sulfide-containing environment because they need this substance which they oxidize to sulfur, the oxidation process furnishing the catabolic energy which the more common organisms derive from the oxidation or fermentation of organic materials. Experiments showed that, when sulfide is not available, the sulfur bacteria can maintain themselves by oxidizing the stored sulfur to sulfate.

The studies on the sulfur bacteria (1, 2) were soon followed by similar investigations on iron bacteria (3), occurring normally in water with ferrous salts in solution. The deposition of ferric hydroxide was considered as due to a comparable physiological oxidation process.

The full consequences of the idea that the oxidation of an inorganic substance could provide the energy for vital processes were realized when Winogradsky succeeded in isolating the nitrifying bacteria in pure culture. With these it was shown that the oxidation of ammonia to nitrite, and of nitrite to nitrate, enables the respective organisms to synthesize their cell materials entirely from carbon

dioxide and minerals in darkness (4). This process of carbon dioxide assimilation has been designated by Pfeffer (5) as chemosynthesis, and the organisms as chemosynthetic. More recently the better term chemo-autotrophic has been proposed (6).

Amply corroborated by subsequent workers, the existence of chemo-autotrophic micro-organisms thus became established, and various species have since been isolated which can live and grow in strictly mineral media. The chemical energy derived from the oxidation of sulfide, sulfur, sulfite and thiosulfate, of comparable selenium compounds, of ammonia and nitrite, or of molecular hydrogen, can adequately be substituted for the radiant energy which permits green plants to synthesize organic matter in the light.

Such processes are not restricted to aerobic conditions. Oxidation of sulfur and thiosulfate with the simultaneous reduction of nitrate allows the development of *Thiobacillus denitrificans* in the absence of air (7, 8, 9); similarly certain hydrogen bacteria can grow anaerobically in the presence of nitrate (10). In addition to these chemo-autotrophic processes of nitrate reduction sulfate reduction with molecular hydrogen is also known (10, 11, 12, 13) although decisive experiments to show development of the sulfate reducing bacteria in mineral media, on the basis of oxidation of hydrogen with sulfate, have not yet been carried out. Even in the absence of oxygen, nitrate, or sulfate, hydrogen gas may lead to bacterial development, as during the conversion of hydrogen-carbon dioxide mixtures to methane (14) or acetic acid (15).

In general, it can therefore be said that chemical reactions between inorganic substances, which take place with an increase in entropy, can be used by micro-organisms as a source of energy with which the synthesis of organic matter from carbon dioxide is possible.

The early studies with pure cultures of chemo-autotrophic bacteria led to another discovery which emphasized the physiological peculiarity of these organisms. It was again Winogradsky who established that the nitrifying bacteria are adversely affected by the presence of organic compounds, low concentrations inhibiting growth, higher ones even preventing their metabolic activities. Comparable results were later reported for some of the sulfur bacteria (8, 16). The inability of nitrifying and sulfur-oxidizing bacteria to utilize organic substances has often been contested, but the more careful investigations of this problem (17, 18, 19) have sustained Winogradsky's claims.

Nevertheless, it has been recognized from the beginning that the hydrogen bacteria can all be grown equally well in the absence of hydrogen, at the expense of organic matter. Also certain *Thiobacillus* species can develop normally in organic media, even in the absence of oxidizable sulfur compounds (8, 19, 20, 21). While these findings made it impossible to apply Winogradsky's rigorous definition of the physiological characteristics of "inorgoxidants" (22)—which included the inability to decompose organic substances—to all chemo-autotrophic bacteria, they did not present a serious obstacle to the formulation of a clear-cut criterion for the latter. As such could be considered any organism capable of development in darkness in a strictly mineral medium, by the oxidation of an

inorganic substance, and the concomitant assimilation of carbon dioxide. This definition eliminated the inclusion of various organisms which, like *Aspergillus niger*, can oxidize elementary sulfur, but only in organic media (23). Oxidations of this type appear more nearly comparable with phytochemical reductions; they represent a side-reaction of doubtful physiological significance rather than the primary source of energy for the organism concerned.

Recent advances have, however, made this definition also rather illusory as a satisfactory and unequivocal means of distinction between chemo-autotrophic and heterotrophic organisms. A good example of the difficulty is furnished by the hydrogen bacteria. Already Niklewski (10) made the observation that either one of two pure cultures, isolated from profusely growing crude cultures, did not grow by itself in the mineral medium, whereas inoculation of the two types together resulted in excellent growth. From unpublished experiments of Dr. M. Doudoroff (personal communication) the explanation of such a "symbiotic" relationship appears to be that each type of these hydrogen bacteria requires one or more organic growth factors, produced in excess by the other kind, a situation very similar to the "symbiosis" of certain yeasts and molds which will grow in a mineral medium with sugar when inoculated together, although neither one appears capable of such development by itself (24). This has been shown to be due to the fact that one of the symbionts needs the pyrimidine part of the thiamin molecule for growth, which is produced in excess by the other partner, while the latter requires the thiazole moiety, furnished in turn by the former.

In the case of the hydrogen bacteria it is evident that the combination of the two types can be designated as chemo-autotrophic; either one alone does not correspond to the definition. Although the growth factors for the hydrogen bacteria have not yet been worked out, each one of the two species can be grown in pure culture, and largely by oxidizing hydrogen and reducing carbon dioxide, provided that the medium is supplemented with a small amount of organic matter, the latter serving as a source of supply of one or more substances which the organism needs in a small quantity but which it is unable to synthesize. The question then arises whether this quantitatively insignificant lack of synthetic ability should be regarded as a sufficient reason for excluding the species from the group of chemo-autotrophic bacteria, thus losing sight of its major physiological characteristic, *viz.*, that of being able to synthesize the bulk of its cellular constituents from carbon dioxide with energy derived from the oxidation of an inorganic substance.

It would, of course, be possible to re-define chemo-autotrophism in such a manner that the above difficulty could be avoided, for example by emphasizing the occurrence of a carbon dioxide assimilation in the dark. But at present such attempts also appear doomed to failure. This is due to the discoveries of the past 7 or 8 years that carbon dioxide assimilation or reduction is far from limited to organisms which one might still be inclined to accept, though not in the strictest sense of the word, as chemo-autotrophs. It has been shown that *E. coli* can convert carbon dioxide and hydrogen into formic acid (25); that the

bacterial production of methane is actually a process of complete carbon dioxide reduction (26, 27); that propionic acid bacteria (28, 29), *E. coli* (30), molds (31), and even protozoa (32, 33) and animal tissues (34, 35) can assimilate carbon dioxide. In the last-mentioned cases the assimilated carbon occurs normally in the form of acidic metabolic products, among which the four-carbon dicarboxylic acids are the most important (36, 37). In spite of this genuine carbon dioxide assimilation no one would be willing to apply the epithet chemo-autotrophic to the organisms in question, whose metabolism, under all circumstances, consists predominantly of decomposition processes of organic substances.

At one time the differentiation between an autotrophic and heterotrophic mode of life carried the implication that the former was quite independent of the activities of other living organisms. In a measure this, of course, is true. But considering what the reality of the cycle of matter connotes, this viewpoint is also vulnerable; ultimately the autotrophs now living are as much dependent upon the heterotrophs as *vice versa*. And, fortunately, the important problem is not so much to find an unassailable definition of chemo-autotrophism as it is to reach a better understanding of natural phenomena. Hence we may leave the decision as to whether the above-mentioned hydrogen bacteria and various other organisms with similar properties should properly be called chemo-autotrophs or heterotrophs to those who can make out a good case for its significance. This attitude seems the more justifiable because a number of other facts have tended to cause a merging of the two designations.

2. *Energetic relations in the metabolism of chemo-autotrophic organisms.* The great importance of the chemo-autotrophic mode of life lies in the fact that it admits of a comparative study of the biochemistry of carbon dioxide assimilation. So long as this process was considered as the special prerogative of green plants, it was necessary to regard the photochemical nature of the reaction as essential for the fate of carbon dioxide. It could, therefore, not be decided whether carbon dioxide assimilation was connected with the absorption of radiant energy in a direct or indirect manner. The existence of organisms which could accomplish an assimilation of carbon dioxide in the dark showed conclusively that for the conversion of carbon dioxide into organic matter the absorption of light was not a necessary prerequisite.

In looking for a common denominator of the photo- and chemo-autotrophic assimilation processes, it became clear that a meeting ground could be found by considering the energetic relations. In both cases one could point to a source of energy by which the synthesis of organic substances from carbon dioxide was made possible. To the photosynthetic organisms the energy was obviously supplied by absorbed light quanta, while to the colorless chemo-autotrophs it was furnished by an exergonic chemical reaction.[1]

Thus it becomes understandable that studies on the energy relations were among the first which were carried out with chemo-autotrophic bacteria. Initially such investigations dealt with a determination of the quantity of inorganic

[1] The term "exergonic" is used in preference to "exothermic." See C. D. Coryell, Science **92**: 380, 1940.

substrate that was oxidized and of the amount of organic matter meanwhile synthesized by the organisms. The application of thermodynamic data to the results then gave rise to computations of the "efficiency" of the processes, and to a comparison with that of the photosynthetic reaction. Since this particular phase has been reviewed in a previous volume of this Journal (38), it will suffice to summarize the results. As a rule, the efficiency of a variety of types of chemo-autotrophic metabolism was found to be of the same order of magnitude (± 7 per cent). The hydrogen bacteria, as an exception, appeared noticeably more efficient; from Ruhland's data (39) on the ratio of hydrogen oxidized to carbon dioxide assimilated an efficiency of over 25 per cent was calculated. It was further stressed that none of the chemo-autotrophic processes even approaches the efficiency of photosynthesis which, according to the results of Warburg and Negelein and of Wurmser, is around 80 per cent.

In the light of recent studies of the photosynthetic reaction this comparison can no longer be considered valid; the high efficiency values have not been substantiated. First, Arnold (40) and Manning *et al.* (41), using different methods of experimentation, arrived at considerably lower values (± 35 per cent). The discrepancy with the earlier data has been satisfactorily accounted for by the fine work of Emerson and Lewis (42), in which the manometric measurements of Warburg and Negelein could be fully corroborated, but which also showed that the method of computation of the efficiency was incorrect because the underlying assumption that the gas produced during brief periods of illumination is exclusively oxygen is contrary to the facts. At present, therefore, the best values for the efficiency of photosynthesis must be accepted as well below 40 per cent.

Secondly, there is a good reason for maintaining that this high efficiency measured during photosynthesis is not really comparable with the figures computed by Becking and Parks for chemo-autotrophic processes. The former is based upon metabolism studies of short duration so that one may reasonably expect that the carbon dioxide assimilation has resulted chiefly in the formation of the primary assimilation product, carbohydrate. In addition, the assimilation values are always corrected for the simultaneously occurring respiration. The data on chemo-autotrophic organisms, on the other hand, are derived from experiments in which the extent of carbon dioxide assimilation was determined by measuring the organic carbon content of cultures in mineral media after a period of growth of the bacteria often extending over weeks. Consequently the assimilation process must have gone far beyond a primary assimilatory product; all the different cell constituents, ultimately derived from the primary product by secondary reactions, are included in the final determination. It will be evident that such secondary processes, as, for example, the formation of lipids, may involve quite considerable losses. Furthermore, corrections for possible respiration processes in which organic compounds are oxidized have not been applied here. Thus it becomes clear that the measured or computed efficiencies in those cases must fall considerably below the maximum values which would represent the

efficiency of the primary carbon dioxide assimilation in a chemo-autotrophic process. The above-mentioned attempts at evaluating the efficiency of chemo-autotrophs have, therefore, not contributed much beyond the demonstration that the over-all conversion of carbon dioxide into the various components of bacterial cells is less efficient than the production of a primary assimilate from carbon dioxide by photosynthesis.

While Ruhland was fully aware that his data did not give an accurate idea of the true efficiency of the carbon dioxide assimilation of hydrogen bacteria and left it at that,[2] Burk (43) believed that in this case an accurate computation of the "thermodynamic efficiency"—as contrasted with Ruhland's "machine efficiency"—could be accomplished by applying to the over-all figures a correction for energy consumption not directly connected with the assimilation process. In this manner the conclusion was reached that this assimilation must be considered as, thermodynamically, completely reversible. On account of its theoretical importance this paper will be considered in some detail, especially because the method of calculation rests upon an assumption which is open to serious doubt.

Ruhland had shown that the hydrogen bacterium, *Bacillus pycnoticus*, when growing in the presence of both glucose and hydrogen, does not appear to oxidize the latter. From this and similar observations Burk drew the inference that the oxygen consumption by these hydrogen bacteria should be ascribed in its entirety to an oxidation process of organic substances, and that oxygen is not used at all for the direct oxidation of molecular hydrogen. Starting with this assumption it then follows that the oxygen utilization in Ruhland's experiments can only be accounted for by an oxidation of organic compounds previously synthesized from carbon dioxide and hydrogen. This would permit of a calculation of the total organic matter synthesized by the bacteria during the experiments by adding the equivalent of the oxygen consumed to the amount formed during growth in a culture in inorganic media. If this is done, the total carbon dioxide assimilation corresponds very closely to that required by the reaction equation:

$$CO_2 + 2H_2 \rightarrow \tfrac{1}{6}C_6H_{12}O_6 + H_2O$$

the slight deviation being due to the fact that the average cell material of the bacteria is slightly more reduced than carbohydrate.

Burk further showed that, since the oxidation of 2 mols of hydrogen can furnish 108460 cal. of free energy, and the reduction of 1 mol of carbon dioxide

[2] "Über die Energieausnutzung dieses Prozesses für die Chemosynthese schlechthin lässt sich nichts aussagen, da der in der Bilanz der letzteren genau entgegegengesetzte Vorgang der Atmung gleichzeitig abläuft, und es nicht gelingt, uns über das während der Assimilation erreichte Mass derselben Klarheit zu verschaffen. Wir können uns vielmehr nur über den für den Körperaufbau nutzbar gemachten Bruchteil der bei der Knallgasreaktion disponibel werdenden Gesamtenergie eine Vorstellung verschaffen, also m.a. W. für die Differenz: organische Stoffproduction minus Betriebsstoffe" (**39:** p. 384).

requires 105140 cal., the theoretical relation for the above process, if it were completely reversible, is:

$$\frac{\text{No. of mols } H_2 \text{ needed}}{2 \times \text{No. of mols } CO_2 \text{ reduced}} = \frac{105140}{108460} = 0.970.$$

The experimental data, treated in the above manner, furnish practically the same result:

$$\frac{\text{No. of mols } H_2 \text{ used}}{2 \times \text{No. of mols } CO_2 \text{ reduced}} = 0.966 \pm 0.012,$$

from which follows the conclusion that the reduction of carbon dioxide is indeed a perfectly reversible process.

This argument seems, however, fundamentally deficient, and amounts to proving an assumption by taking its correctness for granted, because in the original assumption the outcome of the computation is already implied. This follows from a simple analysis.

The culture of hydrogen bacteria growing in a mineral medium in the presence of hydrogen contains as oxidizable materials only hydrogen and the organic matter of the bacterial cells. The consumption of oxygen must, therefore, be due to an oxidation of A, molecular hydrogen; B, organic matter, or C, both. Since meanwhile organic matter is formed from carbon dioxide, case A can be represented by the set of equations:

$$n\,[2H_2 + O_2 \rightarrow 2H_2O] \quad \ldots\ldots A_1$$

$$CO_2 + 2H_2 \rightarrow (CH_2O) + H_2O \quad \ldots\ldots A_2$$

$$2(n+1)H_2 + nO_2 + CO_2 \rightarrow (2n+1)H_2O + (CH_2O)^3 \quad \ldots\ldots A_3$$

Similarly, case B corresponds to:

$$(n+1)[2H_2 + CO_2 \rightarrow (CH_2O) + H_2O] \quad \ldots\ldots B$$

$$n[(CH_2O) + O_2 \rightarrow CO_2 + H_2O] \quad \ldots\ldots B$$

$$2(n+1)H_2 + nO_2 + CO_2 \rightarrow (2n+1)H_2O + (CH_2O) \quad \ldots B_3 = A_3$$

while case C is a combination of A and B.

The analytical data concerning hydrogen, oxygen and carbon dioxide utilization and organic matter production in Ruhland's experiments obviously apply only to the final, overall equations. And, since these are identical for all three cases, a decision as to which reactions occur must be based upon other considerations. Now, the assumption that all oxygen, consumed during the growth of the organisms, has been used for the oxidation of (CH_2O) implies that the hydrogen can only have reacted according to equation B_1. That a computation on this premise leads to the theoretical value of the entropy relations is,

[3] In these equations (CH_2O) designates the assimilation product, not formaldehyde. Though an approximation, it is sufficiently accurate for the purpose, and has the advantage of simplicity.

therefore, obvious. Burk's results could thus have been anticipated. But they prove nothing beyond the fact that Ruhland's analyses have been accurate.

It has already been mentioned that all known hydrogen bacteria can live heterotrophically. As such they are capable of oxidizing a great variety of organic compounds. It could, therefore, be expected that in the presence of both hydrogen and an organic substrate the oxidation of the former is suppressed. Furthermore, the inability of bacteria to oxidize hydrogen when grown in organic media is often due to selection, adaptation, or both. Hence the observations of Ruhland on the inhibition of hydrogen oxidation by glucose do not justify the assumption that autotrophically growing hydrogen bacteria use oxygen only for the oxidation of organic substrates.

In three recent reports (44, 45, 46) on the oxidation of molecular hydrogen by hydrogen bacteria, purple bacteria, and green algae it has been pointed out that in all cases this reaction is dependent upon the presence of carbon dioxide. This finding may seem to strengthen Burk's assumption which would naturally require the presence of carbon dioxide for the utilization of hydrogen. It must, however, be borne in mind that also for the oxidation of organic substances the presence of carbon dioxide is a prerequisite (47) and here the explanation for this phenomenon must evidently be quite different (37). Thus it is readily conceivable that the reason for the necessity of carbon dioxide in the oxidation of hydrogen would be the same as in the oxidation of organic compounds.

3. *Biochemical mechanisms in the chemo-autotrophic bacteria.* As long as the link between chemo-autotrophic and photosynthetic carbon dioxide assimilation was considered to be principally an energetic one, the approach outlined in the previous section was the most promising. It could have been refined by attempts at obtaining a clearer separation of the primary assimilation process from secondary conversions, for example by conducting experiments of short duration. In addition, careful measurements of an endogenous respiration process, both before and after a period in which an inorganic substrate was being oxidized, might have permitted the application of a satisfactory correction for the destruction of organic matter during the assimilation process proper. Such refinements would have yielded data on the efficiency of the assimilation more directly comparable with those available for photosynthesis. However, the interest in a comparative study of the two assimilation processes had meanwhile begun to move in a different direction.

Where energy from absorbed light quanta or from exergonic chemical reactions is used for the conversion of carbon dioxide into organic matter, there must exist a mechanism for the transfer of energy. The gradual development of a better understanding of the chemical mechanisms involved in catabolic processes, both oxidative and fermentative, had been spectacular, and toward the end of the 1920's a clear enunciation of the major principles of these mechanisms had been achieved. Briefly, these principles can be summarized as follows:

1. Any biochemical process can be considered as consisting of a series of individual step-reactions.

2. Each one step constitutes a chemically intelligible, and extremely simple type of reaction.

3. The common property of all such reactions is that they can be regarded as examples of hydrogen (electron) transference from one constituent to another.

4. Thermodynamically each step is a spontaneous (exergonic) reaction.

The most general expression of these principles was given in Kluyver's well-known reaction equations (48):

$$AH + B \rightarrow A + BH\cdot$$
$$AH\cdot B \rightarrow A\cdot BH$$
$$AH\cdot B \rightarrow A + BH$$
$$AH + B \rightarrow A\cdot BH$$

It is understandable that the fruitfulness of these concepts of the intimate mechanism of catabolic processes led to a desire to apply them to those biochemical reactions which are known as anabolic. Since the conversion of carbon dioxide into organic matter is quantitatively and economically the most important instance of the latter, early attempts were made to formulate possible step-reactions for this process which would be in harmony with these ideas.

Photosynthesis soon appeared amenable to such an interpretation, at least in part, when it was found that certain colored bacteria, the green and purple sulfur bacteria, carry out a reduction of carbon dioxide which is dependent upon both illumination and the presence of oxidizable substances, and which proceeds without the evolution of molecular oxygen (49, 50). These results led to a generalized formulation of the photosynthetic process as rendered by the equation

$$CO_2 + 2H_2A \xrightarrow{\text{Light}} (CH_2O) + H_2O + 2A.$$

From this equation it would appear that green plant photosynthesis represents a special case in which H_2O serves as the hydrogen donor, H_2A, which is dehydrogenated to molecular oxygen.

It thus became possible to compare the features of various types of carbon dioxide reduction in the hope of thereby establishing those characteristics which are common to all such processes, and of eliminating secondary, perhaps fortuitous aspects. An approach of this kind, for which Kluyver had coined the name "comparative biochemistry" (48), has frequently yielded remarkably instructive results (see, *e.g.*, (51)).

Such a comparison of the different photosynthetic processes reveals the fact that the common features are the absorption of radiant energy and the reduction of carbon dioxide, while the specific hydrogen donors may differ widely. Since, furthermore, the oxidation of all the possible hydrogen donors, with the sole exception of H_2O, can also be accomplished by the respective organisms in the dark with the utilization of oxygen, it appeared as if there would exist a direct link between the carbon dioxide reduction and the absorption of light quanta. But here the existence of chemo-autotrophic organisms warns to caution; their metabolism shows unambiguously that the reduction of carbon dioxide *per se* does not require light. And because, in this group of organisms, we are therefore compelled to admit the existence of a mechanism whereby the conversion of carbon dioxide into organic matter can be accomplished without the co-opera-

tion of radiant energy, our present concepts of "comparative biochemistry" make it tempting to suppose that the same, or at least a closely similar mechanism, may well be operative in photosynthetic organisms too. Some recently established facts make this even more than suggestive.

Among the photosynthetic purple bacteria are certain species which can assimilate carbon dioxide in the presence of molecular hydrogen. These organisms can also be grown in the dark like regular hydrogen bacteria. Instead of carrying out a photosynthetic metabolism they now behave as chemo-autotrophs. A similar conversion of photochemical to non-photochemical carbon dioxide assimilation has been accomplished with certain green algae by Gaffron (46). These observations force us to the conclusion that either two entirely different mechanisms for carbon dioxide reduction exist side by side in the same organism, or that the biochemical mechanism for the assimilation of this compound is always independent of light in these organisms, and that the absorption of radiant energy in photosynthesis is immediately connected with a step-reaction which is somewhat removed from the reduction process itself. At present the tendency is distinctly in favor of the latter alternative. In this line of thought a study of the metabolism of the chemo-autotrophic bacteria naturally becomes all the more important because it may contribute to an elucidation of the mechanism of carbon dioxide assimilation in photosynthesis as well.

Recently some advances of great interest have been made in this direction. Experimenting with the colorless sulfur bacterium *Thiobacillus thiooxidans*, Vogler has found that the assimilation of carbon dioxide, which in growing cultures accompanies the oxidation of sulfur to sulfuric acid, can be separated in time from the energy yielding oxidation process. A suspension of the organisms, supplied with a small quantity of sulfur in the absence of carbon dioxide, rapidly consumes oxygen until the sulfur has been completely oxidized. If at this point carbon dioxide is admitted, a rapid uptake of carbon dioxide is observed. It could, moreover, be shown that this assimilation process is entirely independent of simultaneous oxidation reactions because it occurs, and quantitatively to the same extent, in an atmosphere devoid of oxygen (52). From these results it may, therefore, be concluded that during the oxidation of sulfur, and in the absence of carbon dioxide, the energy released during the process is stored up in the cells and can be used afterwards for the assimilation of carbon dioxide. It is then logical to infer that normally, when the bacteria oxidize sulfur and grow in the presence of carbon dioxide, the assimilation proceeds by the same mechanism.

The discovery that oxidation of sulfur and assimilation of carbon dioxide do not necessarily occur simultaneously, but can be separated in time, suggests strongly that the mechanism of energy storage and release involves the building up of a store of chemical substances with a high energy content upon which the cells can draw at some later time in order to make possible the occurrence of a reaction which by itself is endergonic. And the very fact that the net result can be represented by a reaction equation in which substances other than sulfur, oxygen, carbon dioxide, and the assimilation product do not occur implies that the "high-energy compounds" would be involved in a cyclic change. It then

becomes possible to formulate the chain of events associated with the chemoautotrophic metabolism somewhat more satisfactorily.

Instead of the more conventional representation:

$$\begin{cases} n(2S + 3O_2 + 2H_2O \rightarrow 2H_2SO_4) \ldots\ldots\ldots\ldots \text{Exergonic} \\ CO_2 + H_2O \rightarrow (CH_2O) + O_2 \ldots\ldots\ldots\ldots \text{Endergonic} \end{cases}$$

amplified by the statement that these reactions are energetically coupled, a series of reactions can be postulated as follows:

$$n(2S + 3O_2 + 2H_2O + X \rightarrow 2H_2SO_4 + Y)$$

$$CO_2 + nY \rightarrow (CH_2O) + nX$$

in which both steps are exergonic.

At first sight this modified formulation does not appear particularly useful as long as X and Y remain unspecified entities. It might also, and rightly, be objected that it does not present an unusual or new feature. Physical chemists as well as those biologists who have occupied themselves with studies on the actual mechanism of energy transfer have long felt that the energetic coupling of two reactions requires a chemical mediator. And, if no further evidence as to the nature of X and Y were available, it would indeed be futile to stress this formulation. The point to be made is, however, that there are good reasons to believe that in this respect important information has been gained which not only tends to reveal the nature of the chemical mediators, but also indicates the very general application in biochemical processes of one and the same group of compounds in this capacity.

Vogler and Umbreit (53) have, namely, succeeded in demonstrating the direct coupling of phosphate transformations with both sulfur oxidation and with carbon dioxide assimilation. Evidently this became possible only after the experimental separation of the last-mentioned reactions had been accomplished. Their studies on the amounts of phosphate derivatives in the medium and in the cells had previously shown that in the combined presence of sulfur, oxygen and carbon dioxide significant changes in the phosphate compounds could not be observed. But if *Thiobacillus thiooxidans* was allowed to oxidize sulfur in the absence of carbon dioxide the oxidation was found to be accompanied by the disappearance of inorganic phosphate from the medium, and its conversion, in the cells, into an organic phosphate ester. Conversely, the subsequent assimilation of carbon dioxide, unaccompanied by a simultaneous oxidation of sulfur, results in the concomitant release of inorganic phosphate to the medium. It thus appears that in the assimilation process of *Thiobac. thiooxidans* a phosphate cycle is involved in which the product responsible for the storage and transfer of energy can be identified with an organic phosphate ester. The composition of the organo-phosphates of *Thiobacillus* cells has been elucidated by the recent analyses of Le Page and Umbreit (54), and in a personal communication Umbreit has stated that the energy-storage material has been shown to be adenosinetriphosphate, identical with that from yeast.

The significance of this discovery can hardly be over-estimated. Not only does it permit of a clearer concept of the nature of the terms *X* and *Y*, but it further reveals that the chemical mechanisms for energy transfer are very closely similar in all living organisms, including autotrophic as well as heterotrophic cells. Particularly impressive in this connection becomes the "utility in biochemistry," advocated so convincingly by Kluyver and Donker (55) as early as 1926, to which now the generality of the phospho-organic esters as energy transferring compounds may be added. The important rôle played by these esters in biological syntheses and in the mechanism of muscular contraction have recently been clarified by the important contributions of Cori *et al.*, Hanes, Kalckar, and especially Lipmann (56–59). It now appears likely that also the initial stages of carbon dioxide assimilation in chemo-autotrophs are associated with these substances. And Ruben (60) has just proposed a well-conceived working hypothesis of the mechanism of photosynthesis in which these aspects are incorporated.

Two problems have been opened up by this new development. The first concerns a clarification of the reaction steps in which inorganic phosphate becomes esterified; the second, the establishment of the actual mechanism of the initial carbon dioxide fixation. Regarding the former point nothing can be said as yet with any degree of certainty. In the metabolism of organic compounds phosphate esters arise in which, through dehydrogenation, the phosphate bond energy can become so increased as to represent up to 12,000 calories. Whether similar phosphate esters are possible with inorganic sulfur compounds, or whether the latter themselves are combined with organic phosphate esters, conceivably replacing the phosphates, is unknown.

Also the chemical reactions by which carbon dioxide is converted into an organic substance have not yet been elucidated, although much progress has been made in this respect during the past few years. One of the best established mechanisms is that in which a four-carbon dicarboxylic acid is formed from carbon dioxide and a three-carbon compound, the latter in all probability being pyruvic acid (36, 37, 61, 62). That this mechanism may not be operative in the chemo-autotrophic bacteria seems to follow from the observation of Umbreit *et al.* that here pyruvate in low concentration inhibits carbon dioxide fixation. However, further experiments are needed, and it seems likely that any advances made in this field will immediately reflect on our present concepts of carbon dioxide assimilation by any one organism, whether photosynthetic, chemo-autotrophic, or heterotrophic. It is becoming increasingly evident that all biochemical reactions follow similar general pathways, so that a better understanding of one is soon followed by direct applications to other processes.

4. *Autotrophic organisms and the metabolism of organic matter.* One aspect of the transformation of carbon dioxide into cellular materials remains to be discussed. This concerns the means whereby the great variety of cell constituents are formed. Two main possibilities offer themselves as working hypotheses. The first is that each substance is produced more or less directly from carbon dioxide and whatever other minerals enter into its composition. The alternative

would be the assumption that only one or a very few organic substances result from the process of carbon dioxide assimilation *per se*, and that the different and manifold components which together constitute the cell originate by a series of secondary transformations of such primary assimilation products. From the point of view of "comparative biochemistry" the latter hypothesis seems infinitely more probable and logical. This, however, implies that the organism so operating must be capable of carrying out conversions of organic compounds. Theoretically it should even be able to grow heterotrophically on the proper organic substrates. Many of the photo-autotrophic organisms have yielded to this treatment; also a number of genuine or presumed chemo-autotrophic organisms, such as hydrogen bacteria, certain sulfur bacteria, and iron bacteria, can develop satisfactorily in organic media in the absence of the specific oxidizable inorganic substance. But there remain, as apparently insuperable obstacles, those cases which seem to fit Winogradsky's most rigorous definition of the chemo-autotrophs *par excellence*, *i.e.*, organisms which, at best, cannot utilize organic compounds, and are often harmed by them. Clearly, if autotrophs exist which cannot metabolize any organic material, this fact alone would suffice to disprove the second possibility.

However strong and convincing the arguments in favor of the occurrence of such organisms may have seemed, such claims can no longer be considered conclusive. On the contrary, a number of recent studies have shown that the experimental evidence points decisively in the opposite direction. First, Cataldi has successfully cultured *Beggiatoa alba*—for which Keil (16) had maintained the ineffectiveness of organic substances, and the utilization of carbon dioxide as only carbon source—in sulfide-free organic media (63). Secondly, also the iron bacteria of the genus *Leptothrix* are, in pure culture, not dependent upon the presence of ferrous ions but can grow at the expense of organic matter (64). Finally, even the repeatedly substantiated reports that the nitrifying bacteria, as well as certain *Thiobacillus* species, cannot metabolize organic substances have been refuted. Boltjes (17) had observed a favorable influence of "Nährstoff Heyden," an egg-albumen product, and some of the fatty acids on the growth of nitrifying bacteria. Later, Bömeke (65) showed that, in the absence of ammonia and nitrite, these organisms actually carry out an oxidation of organic substances. The occurrence of a similar process in *Thiobac. thiooxidans* has also been proved (66, 67). It must, consequently, be conceded that even these most typical of chemo-autotrophic bacteria have failed to furnish experimental facts which would rule out the hypothesis that the synthesis of cellular materials proceeds by way of secondary conversions of one or a few primary assimilation products. It is true that the above experiments with nitrifying bacteria and *Thiobac. thiooxidans* have only shown that previously synthesized organic matter can be oxidized by these bacteria themselves. Also, the rate at which such oxidations occur is very low. Attempts at increasing this rate by the addition of various organic substances which might function as metabolites have, so far, not been promising. Nevertheless, it seems well within the realm of possibility that future work in this direction may establish the existence of specific types of

organic compounds or of the need of particular environmental conditions to accomplish this end. Then it might ultimately become possible to provide the organisms with an organic medium, devoid of oxidizable inorganic energy sources, in which development can be secured. It should, however, be emphasized at this point that a successful achievement of this program must not be interpreted to mean that, under natural conditions, one could find the organisms growing heterotrophically wherever the proper organic materials and conditions are present; ecological considerations show that in nature their development and distribution would be restricted to those environments where the specific inorganic substrate occurs.

The ability of the nitrifying bacteria to oxidize organic materials has suggested to Bömeke that this function might explain the wide discrepancies in previous determinations of the ratio ammonia (nitrite) oxidized to carbon dioxide assimilated. For if a culture were analyzed some time after the oxidation of the inorganic substrate had been completed a considerable fraction of synthesized organic matter might meanwhile have been re-oxidized. It cannot now be decided for which experiments this would hold true, but it is certain that future work on this phase must pay close attention to this possibility.

At first sight, Ruhland's data on the hydrogen bacteria appear to contradict this idea because the ratio hydrogen oxidized to carbon dioxide reduced is definitely not a function of time. However, the unusual experimental conditions provide a satisfactory answer to this situation. In all cases the amount of hydrogen in the cultures was considerably in excess of the available oxygen, so that the bacteria were never exposed to conditions where oxidation of cell materials could supersede the chemo-autotrophic reactions for lack of oxidizable inorganic substrate. This may partly account for the high efficiency values observed with the hydrogen bacteria.

In addition to the just discussed uncertainty attaching to the evaluation of the previously determined substrate—carbon dioxide ratios, another factor must also be considered. It has already been remarked that in long-time experiments the oxidation of an inorganic substrate will be accompanied by a further conversion of a primary assimilation product which may entail appreciable losses. In that event all previously established values for such ratios are low. In view of what has been said earlier in connection with the metabolism of the hydrogen bacteria (p. 344–345) it is clear that even the simultaneous determination of substrate, oxidation product, oxygen consumption, and carbon dioxide assimilation could not overcome this difficulty. At present the closest approximations could be achieved by short-time experiments in which due consideration is given to experimentally determined corrections for the concomitant oxidation of organic substances.

5. *The evolutionary significance of chemo-autotrophic organisms.* The discovery of chemo-autotrophic bacteria showed that living organisms can exist and grow in a mineral environment and in the absence of light. The assumption that such a combination of factors occurred on earth when the surface had cooled down far enough to permit the existence of living beings has led to specu-

lations in which the chemo-autotrophic bacteria were considered as the most "primitive" of all known organisms. The most interesting reconstruction of an evolutionary scale among microbes is unquestionably Orla Jensen's attempt (68), based upon the consistent use of such biochemical premises. In this and similar schemes it is taken for granted that, at the time when living organisms could start inhabiting the earth, organic matter must of necessity have been lacking. Recently, however, Oparin has exposed the fallacy of this premise (69), and pointed out that the synthetic mechanisms of the known autotrophic organisms in general appear so much more complex than those of heterotrophs that, on biochemical grounds, one could make out a good case for the thesis that the most primitive of bacteria must have belonged to the latter category. The simple nutrient requirements of the chemo-autotrophs certainly do not justify the conclusion that hence their metabolism is simple!

Metabolism is governed by enzyme systems, and many of the known enzymes contain active groups which have been identified with vitamins and growth factors (51, 70). It follows that a knowledge of the vitamins required or synthesized by an organism may make it possible to draw inferences as to its metabolic activities. Although only a single investigation on the synthesis of vitamins by strictly chemo-autotrophic bacteria has been published to date, the results are of great interest in that they substantiate the supposition that there exists a close similarity in the metabolism of autotrophic and heterotrophic organisms. O'Kane (71) has established that *Thiobac. thiooxidans*, grown autotrophically in a sulfur-containing medium, contains thiamin, riboflavin, nicotinic and pantothenic acids, pyridoxin, and biotin, *i.e.*, practically the full complement of B-vitamins whose function in the metabolism of organic compounds has either been elucidated or can be readily surmised. This fact militates strongly against considering the metabolism of these bacteria as "primitive." The ability of all known hydrogen bacteria and many other potentially autotrophic organisms to develop with a variety of organic compounds makes it very likely that in these species the conditions would not be materially different. The peculiarities in the metabolism of the nitrifying bacteria make the latter a tempting group of organisms for an extension of this type of investigation.

If, therefore, as the present trends indicate, organic compounds were present before life appeared on the earth, one can readily subscribe to Oparin's further deductions to the effect that purely chemical interactions between such substances may have resulted in the formation of a number of biocatalysts before a wholesale destruction of organic matter by living microbes became possible, and that, consequently, the earlier conditions may have been more conducive to the development of heterotrophic than of autotrophic bacteria.

On the other hand, it must be realized that the foregoing statements do not necessarily imply that the metabolism of the heterotrophic micro-organisms is the more primitive. It becomes increasingly evident that the fundamental biochemical activities which the now living organisms display are found universally distributed. This makes it impossible to infer the course of evolution from such general biochemical considerations.

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INTERRELATIONS BETWEEN THYROID FUNCTION AND VITAMIN METABOLISM

VICTOR A. DRILL[1]

Department of Physiology, Northwestern University Medical School, Chicago

During the past fifteen years considerable literature has accumulated on the interrelationships between the function of the thyroid gland and vitamin metabolism. Most of these studies are experimental, although some clinical observations have been reported. The experimental observations can be divided into two main groups: 1, the effect of a specific vitamin deficiency on the thyroid gland; 2, the effect of administration of a vitamin on animals which were given desiccated thyroid, or injected with thyroxin, or with the thyrotropic hormone of the anterior pituitary gland.

Hyperthyroidism, both experimental and clinical, has been shown to increase the requirements for certain vitamins. This increased vitamin requirement should not imply an antithyrogenic effect, although the increased requirements for certain vitamins indicate that some of the symptoms of hyperthyroidism, previously seen in animals and man, may be due to a secondary deficiency of these vitamins. The administration of these vitamins in larger amounts will correct these secondary changes, as will be pointed out in certain cases. An actual antithyrogenic effect has only been definitely shown in a few cases, although other suggestive evidence is available, which awaits confirmation and elucidation.

THYROID GLAND AND VITAMIN A. *1. Effect of deficiency of vitamin A on the thyroid gland.* McCarrison (1931) found hypertrophy of the rat thyroid to occur during vitamin A deficiency. The thyroid gland of rats and guinea pigs has also been reported to be enlarged and inactive as a result of vitamin A deficiency (Mitzkewitsch, 1934; Uotila, 1938; Schulze and Hundhausen, 1939a). However, other authors were unable to find thyroid hypertrophy during vitamin A deficiency (Drennan, Malcolm and Cox, 1931; Sampson and Korenchevsky, 1932; Sure, 1938). Sampson and Korenchevsky did, however, observe hypertrophy of the thyroid gland when the diet was deficient in both vitamin A and iodine.

Coplan and Sampson (1935) have studied this problem in the greatest detail and with adequate control observations. Their studies show the important influence of sex, time interval and diet in studying such problems. They found that iodine deficiency alone produced thyroid hypertrophy in male and female rats, which persists longer in the female, and is followed by atrophy in both sexes. Vitamin A deficiency produced a definite hypertrophy in female, but consistent atrophy in male rats. Whereas iodine deficiency and vitamin A deficiency produced hyperplasia of the epithelial cells of the thyroid gland, vitamin A deficiency also produced degeneration of the epithelial cells, which the authors

[1] National Research Council Fellow, 1942–1943. Present address, Dept. of Pharmacology, College of Physicians and Surgeons, New York.

believe is a specific effect of vitamin A deficiency. A deficiency of both iodine and vitamin A produced hypertrophy in male and female rats followed by atrophy in the male. Harris and Remington (1939) observed that neither vitamin A nor carotene influenced the utilization of minimal amounts of iodine in the rat.

2. Effect of vitamin A administration on the thyroid gland. As early as 1923 McCarrison found that the administration of cod liver oil would delay the metamorphosis of tadpoles receiving a high iodine intake, and later carotene was reported to exert a similar effect in delaying the metamorphosis of axolotls (Rokhlina, 1936). It was also found that vitamin A would depress the accelerating effect of thyroxin on the metamorphosis of tadpoles (Eufinger and Gottleib, 1933, 1935) and of salamander larvae (Fleischmann and Kann, 1936). These results demonstrate an antagonistic relationship between vitamin A and the thyroid gland in amphibia.

Harvey (1927) found that feeding cod liver oil, which contained some iodine, to goats increased the amount of iodine in the milk more than an equivalent amount of iodine as potassium iodide. The administration of large amounts of vitamin A was reported by Sherwood, Toth and Carr (1934) to reduce the amount of colloid in the thyroid gland of rats, a similar but not as marked a result being obtained with caritol and haliver oil (Sherwood and Luckner, 1935). Uotila (1938) observed that an excess of vitamin A produced a diffuse hyperplasia of the thyroid. Collazo and Rodriguez (1933) also reported exophthalmos in rats receiving large doses of vitamin A. Schulze and Hundhausen (1939a) observed a decreased thyroid activity and decreased thyrotropic content of the anterior pituitary during hypervitaminosis A. According to Wegelin (1939) and Elmer, Giedosz and Scheps (1935) vitamin A does not materially alter the thyroid gland, although in some cases smaller follicles were observed. The addition of 1 per cent cod liver oil to the stock diet of young rats for 4 months significantly reduced the thyroid weight, when compared with rats on the stock diet alone (Freudenberger and Clausen, 1935).

Hypervitaminosis A can be produced in rats by administering 8,000 to 40,000 I.U. vitamin A per day (Drigalski, 1933; Drigalski and Laubmann, 1933; Moll et al., 1933). Fasold and Peters (1933) administered 1 cc. of Vogan[2] per day to rats and produced symptoms of hypervitaminosis A which they reported could be prevented, or cured before paralysis occurred, by administering thyroxin. However, Baumann and Moore (1939) and Weslaw and Wroblewski (1939) could not confirm these results in fact, they both found that the symptoms of hypervitaminosis A were enhanced by injections of thyroxin.

3. Effect of vitamin A on weight loss of hyperthyroid animals. In an extensive series of studies Abelin (1930a, 1930b; Abelin, Goldener and Kobori, 1926; Abelin, Knochel, and Spichtin, 1930) showed the beneficial effects of yeast, egg yolk, cod liver oil, high protein and high fat diets on the weight of rats fed thyroid gland. These authors were interested in finding a beneficial diet for use

[2] Vogan, used extensively in German literature; 1 cc. contains about 6 gamma of iodine, 40,000 rat units of vitamin A, vitamin D potency not stated.

during hyperthyroidism and not in the effect of any particular compound. Von Euler and Klussman (1932) found that rats injected with thyroxin lost weight less rapidly when carotene was administered each day. According to Loumos (1934) a daily dosage of 10,000 U. S. P. units of vitamin A administered to rats receiving 100 mgm. of thyroid gland per day had little effect on the loss of weight. An initial loss of weight was prevented for a few days after which time the loss of weight continued at about the same rate as control rats. Others have observed that vitamin A, administered in various forms, will affect the loss of weight of hyperthyroid animals to various degrees, either only partially or nearly completely preventing the loss of weight (Abelin, 1935; Schneider and Widmann, 1935; David, 1938; Steffan and Zois, 1938). Vitamin A in arachis oil, or arachis oil alone would prevent the toxic action of thyroxin (Fasold and Peters, 1933). These results also depend to a large degree on the dosage of thyroxin or thyroid gland employed, and the age and sex of the rats used. Rats weighing over 140 grams will rapidly lose weight when 100 mgm. of thyroid gland are fed per day, whereas rats averaging 100 grams will gain weight for a while. Also, 100 mgm. of thyroid gland per day will produce a more rapid loss of weight than will an injection of 100 gamma of thyroxin each day, and will thus offer a more rigorous method of testing the effect of vitamin A on weight loss. The sex differences in hyperthyroid rats can be seen from the data presented by Drill (1938b) and Logaras and Drummond (1938). The latter authors administered 19 doses of 100 gamma of thyroxin to rats over a period of 28 days. With thyroxin, and without vitamin A in the diet, their male rats lost an average of 11.0 grams in weight, and with 3000 I.U. of vitamin A per day the hyperthyroid male rats lost an average of 8.6 grams. In the meantime normal male rats receiving 3000 I.U. vitamin A per day gained an average of 21.0 grams. On the other hand, female rats injected with thyroxin without vitamin A in the diet lost only an average of 1.6 grams, whereas hyperthyroid female rats receiving 3000 I.U. vitamin A per day gained an average of 2.6 grams. Normal females receiving the same amount of vitamin A gained an average of 7.0 grams. Their other experiment with rats receiving 10 I.U. vitamin A per day showed a similar result. Although vitamin A may have some protective effect, under specific conditions, against the loss of weight of hyperthyroid animals, it is not as great as generally interpreted in the literature. The slight effect of vitamin A on weight reported by Logaras and Drummond was obtained by the use of small doses of thyroxin. Sure and Buchanan (1937b) showed an increased requirement for vitamin A during hyperthyroidism, as judged by the appearance of xerophthalmia, but did not obtain any effect of moderate doses of vitamin A on the loss of weight of the rats.

According to Weslaw and Wroblewski (1939) rats injected with thyroxin survived longer if vitamin A (as Vogan) was administered. However, they reported an equally good effect by administering sesame oil, the solvent for the vitamin A in Vogan. Thus the effect seems non-specific due to the administration of fat. In fact, the authors state the rats receiving sesame oil were in better condition, had better growth curves and survived longer than those fed the vitamin A supplement.

Wegelin (1939) found that whereas thyroxin increased the number of mitotic figures in the liver, this increase could be prevented by vitamin A. An excess of vitamin A is also reported to reduce the protective action of thyroxin against the toxicity of acetonitrile in the Reid-Hunt reaction in mice (Fleischmann and Kann, 1936). Greaves and Schmidt (1936) obtained evidence of an increased utilization of vitamin A in the hyperthyroid rat. In the thyroidectomized rat the requirement for vitamin A was decreased.

4. Thyroid activity and storage of vitamin A. Euler and Klussman (1932) obtained a reduction of vitamin A and carotene in the liver when thyroxin was administered. When guinea pigs were fed a vitamin A free diet, the administration of carotene led to the storage of vitamin A and carotene in the liver, but when both carotene and thyroxin were given the liver failed to show storage of vitamin A and carotene (Abelin, 1933; Fasold and Peters, 1933). Although Schneider and Widmann (1935) found no carotene and little vitamin A in the liver of hyperthyroid rats, they obtained an increase in liver carotene in thyroidectomized guinea pigs injected with thyroxin. If this report were confirmed it might mean that in the absence of the thyroid gland, even though thyroxin was injected, carotene could not be metabolized to vitamin A. According to Schneider and Widmann (1934) the injection of thyrotropic hormone will also lower the vitamin A stores in the liver of rats and guinea pigs. Logaras and Drummond (1938) found no reduction of hepatic vitamin A in rats injected with thyroxin, although the amount of thyroxin used was small. The administration of thyroxin to scorbutic animals did not cause a reduction of hepatic vitamin A (Balassa and Szanto, 1938). Also, feeding large doses of vitamin A, Baumann and Moore (1939) did not observe any reduction of vitamin A in the liver when thyroxin was injected.

In human cases of exophthalmic goiter normal values or an increase in hepatic stores of vitamin A have been reported. Wolff (1932) observed an increase in hepatic vitamin A in this disease, the mean value for 5 cases of exophthalmic goiter being 210 I.U. per gram, whereas in accidental death the value was 147 I.U. per gram. A similar result was obtained by Moore (1937), four exophthalmic patients showing an average of 304 I.U. per gram of liver, the values after accidental death being 210 I.U. per gram.

5. Effect of vitamin A on carbohydrate and protein metabolism in hyperthyroid animals. Abelin, Knochel and Spichtin (1930) reported that vitamins A and D would prevent the fall of muscle and liver glycogen of rats generally produced by thyroid feeding, and a similar result was obtained with carotene (Abelin, 1935). David (1938) also reported that vitamin A tended to reduce the carbohydrate changes of hyperthyroid animals to a minimum. Carotene was able to counteract the decrease of liver glycogen produced by thyrotropic hormone in guinea pigs but vitamin A did not prevent the disappearance of liver glycogen when thyroxin was administered (Schneider, 1934; Schneider and Widmann, 1934). Microscopically, Wegelin (1939) showed some effect of vitamin A on liver glycogen; whereas the liver glycogen decreased when both vitamin A and thyroxin were administered, it did not altogether disappear, as it did when thy-

roxin was administered alone. However Steffan and Zois (1938) and Fasold and Peters (1933) did not obtain any effect of vitamin A on the liver glycogen of hyperthyroid rats. It was also reported that carotene did not prevent the fall of liver glycogen of thyroidectomized guinea pigs injected with thyroxin (Schneider and Widmann, 1935).

Vitamin A has been shown to lessen the creatinuria seen during hyperthyroidism (Fischer and Oehme, 1933, 1937; De la Bernardie, 1939), and also to prevent the decrease of creatinine in heart and skeletal muscle (David, 1938), but one group of workers were unable to find any effect of vitamin A or carotene on the creatinuria (Steffan and Zois, 1938).

Török and Neufeld (1938) noted that the effect of thyroxin in lowering the serum lipase content of rabbit serum could be prevented by the simultaneous administration of large doses of vitamin A, but the effect was only temporary, for the serum lipase began to fall in a few days, even though the administration of vitamin A was continued.

6. Relation of thyroid function to carotene metabolism. This part of the review is not concerned with any antithyrogenic properties of carotene or vitamin A, but with the effect of thyroid gland function on the conversion of carotene to vitamin A. Noorden (1907) first suggested the association of carotenemia with metabolic disturbances. Since then various authors (Hess and Meyers, 1919; Head and Johnson, 1921; Stoner, 1928) have noted the appearance of carotenemia in certain individuals, and were unable to reproduce this condition by over-feeding of vegetables to control patients. In the meantime Kunde (1926) had noted the appearance of xerophthalmia in rabbits, on a diet that was adequate for normal rabbits, after they had been thyroidectomized for 8 to 12 months. Her result can only be interpreted now to show that in the absence of the thyroid gland a molecule of carotene was not converted into two molecules of vitamin A, with the resultant occurrence of xerophthalmia.

In 1932 Fellenberger and Grueter reported that the milk of thyroidectomized goats remained yellow, which was attributed to the presence of a lipochrome, probably carotene. A year later Fasold and Heidemann showed that thyroidectomy in goats decreased the vitamin A content of goat's milk and increased the carotene content, leading to the conclusion that the thyroid gland is necessary for the conversion of carotene to vitamin A. In the face of this evidence and the work of Kunde it seems certain that in the absence of the thyroid gland carotene is not metabolized to vitamin A, although the work of Kunde should be repeated using synthetic diets with carotene as the only source of vitamin A.

Wendt (1935a) also believes that the thyroid is necessary for the conversion of carotene to vitamin A and for the storage of vitamin A in the liver. Anderson and Soley (1938) reported 13 patients with carotenemia, 5 of which were hypothyroid, 1 hypothyroid case showed hepatic insufficiency, and three other cases showed abnormal liver function. Savy et al. (1938) observed 5 cases of carotenemia with liver damage, and later (1939) one case of carotenemia with hypothyroidism. By studying the dark adaption of hypothyroid patients Wohl and Feldman (1939) also obtained evidence that the conversion of carotene to vitamin

A was disturbed. Escamilla (1942) reported 7 cases and Mandelbaum et al. (1942) reported 1 case of hypothyroidism associated with carotenemia. As thyroid medication was instituted the blood carotene level fell.

Since carotene is a lipochrome its metabolism might be expected to follow the metabolism of other lipochromes during disease. Although carotenemia was observed in hypothyroid patients, some functioning thyroid tissue is still left in these patients. This then raises the question as to cause of the hepatic damage seen in some of these patients. Is it due to a long standing thyroid hypofunction or the effect of an excess of carotene on the liver? Since carotenemia was also seen in patients without evidence of hypothyroidism, but with hepatic damage, it is possible than an abnormal liver function alone may be the cause of the carotenemia. These problems still await experimental elucidation.

7. Effect of vitamin A on metabolic rate during experimental hyperthyroidism. There is a good deal of evidence to show that vitamin A partially counteracts the high oxygen consumption produced by thyroid feeding. Rappai and Rosenfeld (1935) increased oxygen consumption 42.4 per cent by injecting thyroxin, whereas a rise of only 13 per cent was obtained when both vitamin A and thyroxin were administered, and an increase of 10 per cent when carotene and thyroxin were given. Sesame oil had no effect on the B. M. R. Abelin (1935) obtained a similar increase of 50 per cent in oxygen consumption by administering thyroxin and only 20 per cent rise when vitamin A and thyroxin were administered to rats. These results were confirmed on rats by Logaras and Drummond (1938) and Belasco and Murlin (1940). In an acute study on cats, the injection of thyroxin for three days increased O_2 consumption 45.7 per cent, and with carotin and thyroxin the rise was only 25.4 per cent (Smith and Perman, 1940).

The R. Q. of hyperthyroid rats was not affected by vitamin A (Rappai and Rosenfeld, 1935), nor did carotene influence the R. Q. of cats injected with thyroxin for 3 days (Smith and Perman, 1940). This suggests that vitamin A did not selectively affect the metabolism of any particular foodstuff. Belasco and Murlin (1940) also reported that the increase in liver and kidney metabolism seen in hyperthyroidism was not changed by vitamin A.

Chevalier and Baert (1934) reported that vitamin A deficiency increased the B. M. R. of rats, and that large doses of vitamin A decreased the metabolic rate below normal. However, Sherwood, Toth and Carr (1934), Belasco and Murlin (1940) and Sheets and Struck (1942) did not find any affect of vitamin A on oxygen consumption of normal rats. The latter authors also found no significant change in thyroidectomized rats fed large doses of vitamin A. Rappai and Rosenfeld (1935) found that vitamin A lowered the B. M. R. of normal rats 4 to 12 per cent.

Sheets and Struck (1942) confirmed the above reports, finding that after premedication with vitamin A for 50 days thyroid feeding raised the B. M. R. of rats an average of 25.5 per cent, whereas other rats receiving only thyroid showed a rise of 58 per cent. However, after the B. M. R. of these rats had risen to 58 per cent treatment with vitamin A showed a tendency to lower the level, but did not produce any significant change. Thus, while the administration of thyroid

and vitamin A together will prevent the rise in oxygen consumption to the level produced by thyroid alone, there is no experimental evidence to show that treatment with vitamin A will reduce the high B. M. R., once it has been produced by thyroid feeding.

8. Thyrotropic hormone of the anterior pituitary gland and vitamin A. There are reports which indicate that vitamin A will partially prevent the histological changes produced in the thyroid gland of guinea pigs by injection of thyrotropic hormone (Schneider, 1934; Fellinger and Hochstaedt, 1936; Elmer, Giedosz and Scheps, 1935). Parhon and Werner (1935) have reported that thyroxin or thyrotropic hormone reduced the blood carotene in ducks. According to Schulze and Hundhausen (1939a) a lack of vitamin A leads to increased thyroid activity and increased thyrotropic potency of the pituitary gland, whereas hypervitaminosis A has the opposite effect.

9. Clinical studies on thyroid function and carotene and vitamin A metabolism. Mellanby and Mellanby reported (1921b) that patients with exophthalmic goiter receiving a diet high in cod liver oil and low in fat gained weight. Although no figures were given, the B. M. R. was also reported to be lowered. Others have also studied the effect of iodized fatty acid and vitamins A and D in the treatment of Graves' disease (Adamson and Cameron, 1928; Fraser and Cameron, 1929; Rabinowitch, 1929). Their results are complicated by the simultaneous administration of iodized fatty acids or of iodine contained in the cod liver oil.

Wendt (1935a, 1935b) observed that patients with Graves' disease had practically no serum vitamin A and had low serum carotene values. The administration of vitamin A to such patients caused less of a rise in plasma vitamin A than is seen in normal persons. These serum values were observed to increase after successful iodine treatment or surgery. He also found a low serum vitamin A value in cretins, which showed little change when carotene was administered orally. This confirms experimental work discussed above, where the thyroid gland was found to be necessary for the conversion of carotene to vitamin A. Clausen and McCoord (1938) observed two hyperthyroid patients with low plasma vitamin A and carotinoids. The majority of their hypothyroid patients showed a high plasma carotene value, and in all cases the vitamin A value was low. When several of the hypothyroid patients were studied, following thyroid therapy to elevate the B. M. R. and lower blood cholesterol, the carotene and vitamin A values tended to return to normal. Wendt (1935c) also reported that large doses of vitamin A (as Vogan[2]) increased the weight and lowered the B. M. R. from 46 per cent to 20.5 per cent in three hyperthyroid patients, and two other patients showed some improvement (cf. Wendt, 1936). Dietrich (1936) also found vitamin A to be beneficial in three of six hyperthyroid patients. Abelin (1936) advocates the use of vitamin A in conjunction with iodine and diiodotyrosine as an aid in controlling crisis and the temporary exacerbation that may occur under such treatment.

Studies on the dark adaptation of hyperthyroid patients also indicated a deficiency of vitamin A in hyperthyroid patients (Wohl and Feldman, 1939). Evidence was obtained which suggested that the administration of thyroid ex-

tract or a-dinitrophenol facilitates the utilization of vitamin A by the visual mechanism, representing an increase in the speed and extent of visual purple regeneration (Patek and Haig, 1941). Godtfredson (1941) found impaired dark adaptation and liver function in ten hyperthyroid patients. He suggests that the liver damage in hyperthyroidism disturbs vitamin A metabolism.

Fasold (1937) treated 7 girls with adolescent goiter, none showing any symptoms of hypo- or hyperthyroidism, with Vogan, administering 30 drops 3 times a day. After about one month of treatment the tumor had regressed, but recurred a few weeks after treatment was discontinued. The dose of Vogan used supplied about 3.6 gamma of iodine per day.

In 1938 Catel reported a case of hyperthyroidism in a child which was treated with vitamin A. The blood level of vitamin A was raised but the B. M. R. and other symptoms were not affected. Jacobi and Pomp (1938) studied 14 cases of hyperthyroidism, some treated by rest and diet, and others treated by rest, diet and the administration of vitamin A as Vogan. The criteria of improvement they used were decreased B. M. R. and pulse rate, increase in weight, and improvement of general condition, but they did not find any beneficial effects from the vitamin A supplement.

Although experimental evidence shows that vitamin A has some relation to thyroid function, the clinical results are as yet too few to draw any conclusions concerning the efficacy of this treatment, and such factors as hospitalization, diet and rest. It should also be borne in mind that plasma carotene and vitamin A will vary from the norm in diseases other than thyroid disorders (Clausen and McCoord, 1938; Moore, 1937).

Summary. A deficiency of vitamin A will produce thyroid hypertrophy, although a sex difference in response is present. An excess of vitamin A seems to decrease the amount of colloid in the thyroid gland, although this result is still controversial, as is the effect of thyroxin on hypervitaminosis A. In amphibia, however, a definite antagonism between thyroxin and vitamin A has been demonstrated.

Experimental hyperthyroidism in mammals increases the requirements for vitamin A. However, the degree to which the administration of vitamin A will prevent a loss of weight in such animals is not clear, especially as some authors have observed no effect of vitamin A on weight loss and others have obtained the same effect with the vehicle in which the vitamin A is dissolved. The effect of hyperthyroidism on the liver stores of vitamin A, and the effect of vitamin A on liver glycogen during hyperthyroidism still await further clarification, although vitamin A seems to decrease the creatinuria observed in such animals.

All of the evidence to date indicates that in the absence of the thyroid gland carotene is not metabolized to vitamin A. Various authors also agree that the simultaneous administration of vitamin A with thyroxin will partially prevent a rise in the B. M. R., this being the first definite antithyrogenic action of vitamin A to be demonstrated. However, there is no evidence to indicate that the treatment of hyperthyroid animals with vitamin A will lower the B. M. R. There is some evidence to indicate that vitamin A will partially prevent the histological changes in the thyroid produced by the thyrotropic hormone.

Clinically, hyperthyroidism increases the requirement for vitamin A, also lowering serum vitamin A and serum carotene. Although the administration of vitamin A to such patients will provide for the increased requirements, any other effect of vitamin A in such patients awaits further demonstration.

THYROID GLAND AND VITAMIN B_1. *Effect of deficiency of vitamin B_1 on the thyroid gland.* McCarrison (1914) first reported hyperplasia of the thyroid gland of pigeons fed a polished rice diet. Later Spence (1932, 1934) observed thyroid enlargement in rats, the diet however containing multiple deficiencies. There is also said to be a decreased amount of thyroid hormone in the thyroid gland during vitamin B deficiency (Verzar and Vasarhely, 1924) and Aalsmeer (1932) suggests that beriberi is associated with dysfunction of the thyroid gland. According to Fischer (1933) a deficiency of both iodine and vitamin B_1 will produce thyroid hypertrophy. However, iodine deficiency alone will produce thyroid hypertrophy and will reduce the amount of iodine in the thyroid gland.

A recent study by Sandburg and Holly (1933) revealed some interesting results. They fed rabbits an autoclaved diet of alfalfa hay and rolled oats and found that the thyroid increased in size and was very vascular in appearance. They then made the observation that the administration of either iodine or yeast had an effect on the Ca and P metabolism of rabbits with hyperplastic thyroids. Both the iodine and yeast caused a marked increase in Ca excretion, changing the ratio of Ca and P retention from Ca:P = 1:0.3 to Ca:P = 1:216. Presumably the effect of the yeast on the Ca and P metabolism of rabbits with hyperplastic thyroid glands was due to its vitamin B_1 content, as no effect was obtained with autoclaved yeast.

Other experiments on vitamin B deficiency have generally shown an increase in colloid, later, as the time of deficiency increased, followed by atrophy of the thyroid gland (cf. Meyer, 1937, for earlier references,—Schneider, 1938a; Hundhausen and Schultze, 1939). Sure (1938) reported an increase in thyroid weight in B_1 deficiency. No change in the size, iodine content, dry matter, or structure of the thyroid gland of rats on a vitamin B deficient diet has been observed by Carpenter and Sharpless (1937) and Harris and Remington (1939). Carpenter and Sharpless also concluded that the colloid goiter developed in rats by feeding a low iodine and a vitamin B_1 deficient diet could be prevented by feeding more iodine or vitamin B. Harris and Remington failed to confirm this observation. They found no difference between the effect of yeast or autoclaved yeast on the thyroids of rats fed a low iodine diet, and supplements of vitamin B_1 failed to prevent the picture of colloid goiter from appearing. A diminished food intake per se is reported to have no effect on the thyroid gland (Hundhausen, 1939).

Spence (1934) found no effect of a vitamin B extract on size or vascularity of the thyroid gland of rabbits fed fresh cabbage and rolled oats.

Baglioni (1940) reported that the administration of vitamin B_1 did not alter the histological appearance of the guinea pig thyroid, whereas Giedosz (1938a) has reported injections of vitamin B_1 in the guinea pig stimulate the thyroid gland.

2. Effect of deficiency of other B vitamins on the thyroid gland. Giedosz (1938b) observed stimulation of the thyroid in guinea pigs during a deficiency of vitamin

B_2, and Sure (1938) reported that 88 per cent of the rats showed an average increase of 42.8 per cent in thyroid weight during a vitamin B_2 deficiency. Sure also found a slight increase in thyroid weight during a deficiency of vitamin B_6, and also found that 65 per cent of the rats showed a 28 per cent increase in thyroid weight on a B complex free diet. Uotila (1938) reported that a deficiency of the vitamin B complex produced thyroid atrophy, the follicles being enlarged and filled with colloid.

3. Relation of B vitamins to calorie intake and weight during experimental hyperthyroidism. That a relationship exists between calorie intake and vitamin B_1 requirements has been demonstrated by many authors (cf. Cowgill, 1934). Himwich, Goldfarb and Cowgill (1932) first studied the relation of calorie intake to the B vitamins during hyperthyroidism. They found that normal dogs fed a yeast-free diet developed anorexia in an average of 32 days, whereas hyperthyroid dogs fed a yeast-free diet developed anorexia in an average of 17 days. These dogs started to regain their lost weight when 10 grams of a vitamin B concentrate was fed each day. Cowgill and Palmieri (1933) also found that the vitamin B requirement (as yeast) of pigeons was increased by feeding thyroid gland. This report has been amply confirmed (Capraro, 1937; Mouriquand, Morin and Czerszchowska, 1939; Morin and Czerszchowska, 1939).

It was then observed by Sure and Smith (1934) that a potent vitamin B concentrate protected rats receiving thyroxin from a loss of weight. With the advent of crystalline vitamin B_1 Sure and Buchanan (1937a) restudied the effectiveness of vitamin B_1 in *preventing* the loss of weight produced by hyperthyroidism. Their tables show that 7 rats fed diet 3080, containing 15 per cent autoclaved yeast, and receiving 0.2 mgm. of thyroxin (orally or parenterally?) per day gained 64 to 108 per cent of the weight of control rats when a supplement of 30 to 300 gamma of vitamin B_1 was administered each day. Rats on the same diet fed 0.5 gram of yeast per day plus 0.2 mgm. of thyroxin per day gained 52 to 132 per cent of the weight of control rats. The change in weight of untreated rats receiving the same diet and thyroxin is not stated.

Drill (1938b) studied the effect of vitamin B_1 and yeast on hyperthyroid rats that had lost weight, to determine if such treatment would enable them to regain their lost weight. When the rats had lost an average of 23 grams in weight they were injected with 500 gamma of vitamin B_1 per day. The vitamin B_1 stopped a further loss of weight, but the rats did not regain their lost weight until a rich source of the vitamin B_2 complex was administered. A sex difference in response was also observed. These results were confirmed and extended by Drill and Sherwood (1938). They reported that the effect of vitamin B_1 in stopping further loss of weight in hyperthyroid rats was due to the increased calorie intake which it produced. Although vitamin B_1 increased food intake and prevented a further loss of weight, the rats still did not regain their lost weight until a rich source of the vitamin B_2 complex was supplied. Thus in addition to vitamin B_1, some other factors in the B_2 complex are also required in larger amounts than normal. If the rats were limited to 13 grams of food per day the vitamin B_1 injections did not stop a further loss of weight. The effect of vitamin B_1 in pre-

venting a further loss of weight of hyperthyroid rats was confirmed by Peters and Rossiter (1939). It was later found that pyridoxine (vitamin B_6) and calcium pantothenate can effectively replace the B_2 complex addition to the diet of hyperthyroid rats, and the rats regained and maintained their lost weight while still receiving thyroid gland (Drill and Overman, 1942). Thus in addition to vitamin B_1, both pyridoxine and pantothenic acid are required in larger amounts during experimental hyperthyroidism.

When a small amount of yeast was fed to hyperthyroid dogs an initial increase in food intake was observed. The removal of the yeast from the diet after 4 to 9 weeks of thyroid feeding produced a sudden and marked drop in appetite to values below normal within a few days (Drill, 1941). The subcutaneous injection of 2 mgm. of vitamin B_1 restored the appetite to its previous hyperthyroid level within 24 to 48 hours. This suggests that anorexia is not a primary effect of hyperthyroidism but is a secondary symptom due to a deficiency of vitamin B_1.

Hyperthyroid dogs that lost weight, following the removal of the yeast from the diet, require increased amounts of vitamin B_1 and the vitamin B complex in order to regain their lost weight (Drill and Shaffer, 1942), confirming previous work on rats. By administering sufficient B vitamins from the start of thyroid feeding it was found possible to maintain the weight of 3 of 4 hyperthyroid dogs for as long as 112 days. The food intake of such dogs is above normal. A similar maintenance of body weight of thyroid fed rats receiving yeast concentrate was obtained over a period of 79 days (Drill, Overman and Leathem, 1943).

4. Effect of B vitamins on estrous cycle during hyperthyroidism. Earlier studies have shown that thyroid feeding soon produces a constant diestrus in rats. Hyperthyroid rats receiving vitamin B_1 and yeast supplements were observed to maintain normal estrous cycles (Drill and Sherwood, 1938). In further studies it was shown that hyperthyroid rats receiving a normal but small amount of yeast each day, had only two or three estrous cycles before entering complete diestrus. Besides a loss of weight, these rats also showed a fall in pituitary and ovarian weight, ovarian sections suggesting a lack of anterior pituitary hormone. Other rats received thyroid gland and yeast concentrate for 79 days with complete maintenance of weight and estrous cycles. The weights of the pituitary and ovaries were normal, as was ovarian histology (Drill, Overman and Leathem, 1943).

5. The B vitamins and liver function during experimental hyperthyroidism. Youmans and Warfield (1926), using the phenoltetrachlorphthalein test, did not detect hepatic damage in hyperthyroid dogs. With the use of more sensitive tests thyroid feeding was shown to produce an increase in bromsulphalein retention (Drill and Hays, 1940) and a rise in serum phosphatase (Drill and Shaffer, 1943). Drill and Hays (1940) fed thyroid gland to 5 dogs receiving a small but normal amount of yeast. Three of the dogs developed abnormal liver functions during the period of thyroid feeding, the dye retention being further increased by removing the yeast from the diet. The other two hyperthyroid dogs still

had a normal liver function during this period, and removing the yeast from their diet produced an abnormal dye retention within 24 hours. Although the deficiency of the B vitamins seems to bear a causal relationship to the production of hepatic damage in thyroid fed dogs, it does not mean that it is the only or the most important factor. Later studies on this problem were reported (Drill and Hays, 1942), showing that once an abnormal liver function had been produced in hyperthyroid dogs, treatment with large doses of vitamin B_1 and yeast concentrate did not improve the abnormal liver function. It was also shown that a yeast-free diet renders dogs extremely susceptible to the effects of thyroid feeding, an abnormal liver function being produced in an average of 22 days (Drill, Shaffer and Overman, 1943). Thyroid fed dogs receiving a small amount of yeast each day developed an abnormal liver function in an average of 45 days. When hyperthyroid dogs were fed a high B vitamin diet it took an average of 90 days before an abnormal liver function was observed. Thus, the amount of B vitamins fed has a direct relationship to the time elapsed before an abnormal liver develops. A high B vitamin diet will delay but will not prevent the appearance of an abnormal liver function in hyperthyroid dogs.

6. The B vitamins and pulse rate during experimental hyperthyroidism. The effects of diets deficient in vitamin B on the cardiovascular system of man and animals have been discussed by Weiss and Wilkins (1937) and Frazier and Ravdin (1938). In the dog and rat a deficiency of vitamin B_1 produces bradycardia, whereas in human beriberi bradycardia is rarely observed and tachycardia is common. Parade (1938b) reported that thyroid feeding during a deficiency of vitamin B_1 will retard the development of bradycardia and subnormal temperature. In experiments on dogs thyroid feeding produced a marked tachycardia of 150 to 160 beats per minute (Drill and Hays, 1942). The removal of the yeast from the diet of these dogs after 30 to 60 days caused a marked drop in pulse rate to values below 100 beats per minute, while still feeding thyroid gland. The injection of these dogs with vitamin B_1 raised the pulse rate to its previous hyperthyroid level within 24 to 48 hours. Thus, the action of an excess of thyroid gland in producing tachycardia in dogs depends in some manner on an adequate supply of vitamin B_1, possibly through an effect on food intake (Parade, 1937; 1938a).

Hyperthyroid dogs fed a yeast-free diet develop only a slight tachycardia which returns to normal within 10 days (Drill, Shaffer and Overman, 1943), and hyperthyroid dogs receiving a high B vitamin diet maintain a high pulse rate for only 80 to 100 days, after which time the pulse rate falls towards normal. The drop in pulse rate at this time seems to be associated with a compensatory hypertrophy of the heart.

7. The B vitamins and liver glycogen during hyperthyroidism. Abelin and coworkers first studied the effect of foodstuffs on liver glycogen during experimental hyperthyroidism (Abelin, 1930; Abelin, Knochel and Spichtin, 1930). Among other findings, discussed elsewhere in this paper, they found that yeast would prevent the fall in liver glycogen of rats fed thyroid gland for 5 to 6 days. This observation was confirmed by Drill (1937) who studied the liver glycogen of rats

injected with small amounts of thyroxin. Frazier, Brown, and Vars (1939) obtained a partial rise of liver glycogen in one of three groups of hyperthyroid rats treated with yeast and McIver and Winter (1941) obtained maintenance of liver glycogen in 4 of 6 hyperthyroid rats treated with yeast. Drill, Overman and Shaffer (1942) also found that yeast concentrate would maintain the weight and liver glycogen of rats fed thyroid gland for 86 days. Vitamin B_1 is also reported to diminish the creatinuria produced in rats by injecting thyroxin (De la Bernardia, 1939).

8. Effect of hyperthyroidism on vitamin B_1 content of tissues. Rats fed 100 mgm. of thyroid gland per day and 12 grams of a normal diet per day supplying 10.8 I.U. of vitamin B_1 per day showed a normal amount of vitamin B_1 in the spleen, a reduction in the kidney, and a marked reduction in the liver. The ability of hyperthyroid rats to store vitamin B_1 was studied in a similar group of rats injected with 500 gamma of vitamin B_1 during the last nine days of the experiment. When compared with control rats injected with vitamin B_1 the hyperthyroid rats showed a normal amount of vitamin B_1 in the spleen and muscle, a slightly raised content in the heart and a definite reduction in the kidney and liver. The storage in the whole liver was 35.7 per cent less than the controls (Drill, 1938a). Peters and Rossiter (1939) confirmed the above report using rats injected with thyroxin and the caatorulin test for vitamin B_1, and found a lowered amount of cocarboxylase in rat tissues, the greatest lowering again being observed in the liver. Hyperthyroidism in the rat does not increase the rate of absorption of vitamin B_1 from the intestine (Stockholm, Althausen and Borson, 1941).

Schneider and Burger (1938) found that control patients excreted 80 to 100 gamma of vitamin B_1 in the urine per day, being between 7 and 8 gamma per cent, with a serum value of 6.4 gamma per cent. Hyperthyroid patients excreted an average of 8.4 gamma per cent of vitamin B_1 in the urine but with a daily output averaging 224.8 gamma per day. Coincident with this large increase in B_1 excretion was a daily urine output averaging 2770 cc. After operation the urine output sank to an average of 1100 cc. with a normal value of 79.9 gamma of vitamin B_1 per day.

9. Vitamins of the B_2 complex and the thyroid gland. The effect of a deficiency of the vitamins of the B_2 complex on the thyroid gland has been discussed above. In 1926 Kunde reported that thyroidectomized rabbits, receiving the same diet as normal animals, developed pellagra-like lesions on the dorsum of the feet, the neck, mouth and nose, after 8 to 20 months of complete thyroidectomy. It is interesting that Greene (1938) observed two patients with myxedema and pellagra that showed a similarity of gastroenteric, neurologic, and psychic symptoms, and to a certain extent, similar dermatologic manifestations. Drill and Overman (1942), studying the weight changes of hyperthyroid rats, did not find any evidence of an increased requirement of riboflavin, above that supplied by the daily yeast supplement. The increased requirements of pyridoxine and pantothenic acid during hyperthyroidism have been reported above.

10. Thyrotropic hormone of the anterior pituitary gland and the B vitamins.

Elmer, Giedosz and Scheps (1937) did not find any antagonistic effect of vitamin B_1 on the thyroid stimulation produced in guinea pigs with the thyrotropic hormone. Similar results were obtained by Schneider (1936b). Cutting and Robson (1939) reported no effect of daily administration (length of administration?) of 40 I. U. of vitamin B_1 and 12 Sherman units of vitamin B_2 on the B. M. R. of two guinea pigs injected with thyrotropic hormone. Vitamin B_1 did not prevent the death of guinea pigs or lessen the reduction of liver glycogen of guinea pigs injected with large doses of thyrotropic hormone (Schneider, 1938b). Gentzen and Moire (1938) reported that premedication with vitamin B_1 lessens the effect of thyrotropic hormone on the B. M. R. of guinea pigs.

The thyroid gland of rats fed a B_1 deficient diet is said to respond normally to an injection of thyrotropic hormone (Schneider, 1938a), although extracts of the pituitaries of such rats, when tested on immature guinea pigs, were found to be less active than extracts from normal rats (Hundhausen and Schulze, 1939).

Elmer, Giedosz and Scheps (1938), Hoen and Oehme (1938), and Wahl (1939), showed that the administration of riboflavin (vitamin B_2) did not affect the B. M. R. or histological picture of the thyroid of normal guinea pigs, or of guinea pigs injected with thyrotropic hormone. Wahl (1939) reported that riboflavin augmented the increase in B. M. R. produced by thyrotropic hormone. Riboflavin deficiency does not affect the thyrotropic content of the pituitary (Schulze and Hundhausen, 1939).

11. Clinical studies on the B vitamins and thyroid activity. Shimazono (1931) reported that iodine and thyroid substance have a curative effect on the paralysis produced by vitamin B deficiency, but that these substances were not tolerated by patients with beriberi until the cardiac symptoms were first treated and relieved. In studying blood iodine, Takasugi (1931) found that in beriberi there was first an increase in blood iodine and B. M. R. both of which later dropped.

Means (1937) mentioned 3 hyperthyroid patients, studied by Lerman and Hertz, that gained weight when a high B vitamin diet was administered. In 1938 Frasier and Ravdin studied the effect of vitamin B_1 and yeast on the preoperative preparation of patients with Graves' disease. They found not only improved nutritional status, as judged by increased appetite and gain in weight, but also that "patients with severe hyperthyroidism can be adequately prepared for operation in a shorter time". The patients treated with the B vitamins also showed a larger preoperative fall in pulse rate than control patients. No influence of the supplement of B vitamins was found on the B. M. R. or on the severity of the post-operative reaction. Frazier and Ravdin administered about $2\frac{1}{2}$ times the estimated daily requirement of vitamin B_1. Experimental work indicates that the requirement for vitamin B_1 during hyperthyroidism may be larger than this (Drill and Shaffer, 1942), suggesting that larger doses of the B vitamins might be more beneficial. Scrutinio (1939) also reported that administration of 1000 I. U. of vitamin B_1 increased the weight and decreased the B. M. R. of hyperthyroid patients. The author favors the use of vitamin B_1 in cases of hyperthyroidism with cardiac decompensation. Hertzler (1941) also uses vitamin B_1 in preoperative preparation of toxic patients, cardiotoxic

patients, and in thyroid crisis. Jacobi and Pomp (1938) reported on 14 cases of hyperthyroidism; some were treated by rest and diet, while others received the same regimen plus the administration of vitamin A, either alone or in combination with vitamin B_1. They reported no apparent beneficial effects from the administration of vitamin A, either alone or in combination with vitamin B_1. The increased urinary excretion of vitamin B_1 in patients with Graves' disease has been discussed in section 8 of this review.

Summary. As various results have been reported, no definite conclusion can be stated at this time, as to the effect of a vitamin B_1 deficiency of the thyroid gland. However, the experiments of Carpenter and Sharpless, and Harris and Remington show no effect of vitamin B_1 deficiency on the thyroid gland, and in other cases the possibility of an iodine deficiency must be ruled out. Hyperthyroidism increases the requirements for vitamin B_1, vitamin B_6 and pantothenic acid. The administration of sufficient yeast concentrate to thyroid-fed animals will prevent a loss of weight, or will allow hyperthyroid animals which have lost weight to regain their lost weight. The maintenance of body weight is due to the increase in food intake that occurs when the increased requirements for the B vitamins are met. Such animals will also maintain normal estrous cycles.

Experimental hyperthyroidism has been shown to produce hepatic damage, which is related in part to the amount of B vitamins supplied in the diet. Vitamin B_1 also shows a relation to the cardiovascular system in experimental hyperthyroidism, and yeast concentrate tends to maintain the liver glycogen of thyroid-fed animals.

Hyperthyroidism decreases the amount of vitamin B_1 in various rat tissues, and clinically there is an increased excretion of vitamin B_1. Vitamin B_1 does not appear to antagonize the administration of the thyrotropic hormone. Clinically most authors have reported a beneficial effect of vitamin B_1 and yeast in patients with Graves' disease, although one paper reported no effect when vitamin B_1 was administered.

THYROID GLAND AND VITAMIN C. *1. Effect of a deficiency of vitamin C on the thyroid gland.* In 1915 Rondoni and Montagnani observed hemorrhagic infiltration of the thyroid gland of guinea pigs on a vitamin C deficient diet. McCarrison (1920a, b) confirmed this finding and also noted an increase in thyroid weight of scorbutic guinea pigs, sometimes 2 or 3 times normal. An irregular enlargement of the thyroid was also observed by Bessesen (1923). However, Löwy (1923) did not find changes in the thyroid gland of guinea pigs during scurvy, and Meyer (1928) reported irregular changes in the thyroid of guinea pigs fed a vitamin C deficient diet for 30 days and concluded that no noteworthy changes took place.

Harris and Smith (1928) reported an increase in both the height of the follicular epithelium and number of inter-follicular cells, with a decrease in amount and an increase in the vacuolation of the colloid, after *chronic* scurvy lasting 97 days. They also suggested a possible influence of vitamin C on iodine metabolism. Abercrombie (1935) confirmed the findings of Harris and Smith, also observing hemorrhagic infiltration to a slight extent in normal animals, more so

in *acute* scurvy of 29 days' duration, and much more marked in 126 days of *chronic* scurvy. He further found that potassium iodide or thyroid gland, administered during acute or chronic scurvy, exerted their characteristic effect on the thyroid gland, without prolonging the life of the vitamin C deficient guinea pigs, and concluded that vitamin C is not concerned in iodine metabolism. Neither Rondoni and Montagnani, Löwy, Harris and Smith, nor Abercrombie found any effect of starvation on the histology of the guinea pig thyroid.

May (1937) also found that vitamin C deficiency in the guinea pig produced a marked hyperemia, somewhat increased activity, irregular structure, and the formation of utricles and vesicles without colloid. The therapeutic or prophylactic administration of vitamin C would prevent these changes. Other reports on vitamin C deficiency in the guinea pig have also shown hyperplasia and hypersecretion of the thyroid gland, which was more marked in chronic than acute cases (Schulze and Linnemann, 1938; Uotila, 1938). Vitamin C did not prevent the hyperplasia produced by feeding fresh cabbage to rabbits (Spence, 1934).

2. Increased requirements of vitamin C and effect of vitamin C during experimental hyperthyroidism. Whereas the administration of 0.5 mgm. of ascorbic acid per day would prevent the loss of weight of scorbutic guinea pigs, Demole and Ippen (1935) reported that 10 to 20 mgm. per day were required when the animals were injected with 0.1 mgm. of thyroxin per day. Fischback and Terbrüggen (1937b) found that thyroxin administration to scorbutic guinea pigs increased the loss of weight, but that if large doses of ascorbic acid were given before the thyroxin the loss of weight was less. Ray (1938) also reported that the requirements of thyroid-fed rabbits for vitamin C was increased. Vitamin C deficiency and hyperthyroidism are also said to produce the same blood picture in guinea pigs (Aszodi, 1937). Brandt (1937) observed that the effect of thyroid gland on the axolotl was less when vitamin C was also given. It is also reported that vitamin C will reduce the blood iodine of normal and hyperthyroid animals (Lohr, 1936).

3. Effect of vitamin C on carbohydrate and protein changes in hyperthyroid animals. Fischbach and Terbrüggen (1937a) found that the effect of thyrotropin in lowering liver glycogen was inhibited by large doses of vitamin C, but that vitamin C did not prevent the fall in liver glycogen when thyroxin was administered. Large doses of ascorbic acid were also reported to prevent a loss of muscle glycogen and partially prevent a fall of liver glycogen produced by thyroxin (Steffan and Zois, 1938), and would prevent or suppress the loss of muscle glycogen produced in guinea pigs by injecting thyrotropic hormone.

The creatinuria observed during hyperthyroidism has been found to be reduced by the administration of vitamin C (Fischer and Oehme, 1937; v. Plehwe, 1938; Steffan and Zois, 1938; De la Bernardia, 1939). Similar results have also been reported after the administration of thyrotropic hormone (Steffan and Zois, 1938).

Vitamin C will not prevent the fall in serum lipase induced in rabbits by thyroxin (Török and Neufeld, 1938).

4. Thyroid activity and tissue vitamin C. Many authors have reported a decrease of vitamin C in the adrenal during hyperthyroidism (Demole and Ippen, 1935; Mosonyi, 1935; Plaut and Bülow, 1935, Nespor, 1936; Farmer and Fribourg, 1942). Farmer and Fribourg believe that the effect of thyroid feeding in increasing the anaphylactic response of guinea pigs is due to the reduction of adrenal vitamin C, which in turn lowers the cortin content of the adrenal. This hypothesis, however, requires further testing as to the therapeutic administration of vitamin C in preventing the changes reported. Contrary to all of the above reports Paal and Brecht (1937) reported that the administration of thyroxin and thyrotropic hormone to guinea pigs increased the amount of vitamin C in the adrenal, and Ray (1938) observed no decrease in vitamin C in adrenals of thyroid-fed rabbits. Mosonyi (1936) found that the loss of vitamin C in the liver and adrenals of guinea pigs after thyroxin could be prevented by vitamin A. In dogs, hyperthyroidism also reduced the vitamin C content of the liver and adrenals, whereas thyroidectomy produced the opposite results (Thaddea and Runne, 1938). Sure and Theis (1939) observed that the reduction of vitamin C in various rat tissues produced by thyroxin was accentuated when a supplement of vitamin B_1 was not administered.

5. Effect of vitamin C on metabolic rate during experimental hyperthyroidism. Demole and Ippen (1935) and Oehme (1936) found that vitamin C would partially reduce the high B. M. R. seen in hyperthyroidism. This was confirmed by Belasco and Murlin (1940), who also reported that the increased metabolism of liver and kidney during hyperthyroidism was not influenced by vitamin C.

6. Thyrotropic hormone of the anterior pituitary gland and vitamin C. The administration of ascorbic acid is said to produce changes in the thyroid gland similar to that induced by thyrotropic hormone (Heyl, 1934). Schulze and Linnemann (1938) did not find an increase of thyrotropic hormone in the pituitary gland during vitamin C deficiency.

There is some evidence that vitamin C will partially inhibit the action of the thyrotropic hormone in the thyroid gland (Marine, Baumann and Rosen, 1934; Elmer, Giedosz and Scheps, 1935; Schulze and Linnemann, 1938). Other authors, however, did not obtain any effect of large doses of vitamin C on the thyroid hyperplasia produced by thyrotropic hormone (Spence and Scowen, 1935; Schäffer, 1936; Schöber, 1939). Fischback and Terbrüggen (1937a) reported that 20 mgm. of vitamin C per day did not prevent thyroid hypertrophy or loss of weight in animals injected with thyrotropic hormone. Also, the effect of thyrotropic hormone on tadpole metamorphosis was not lessened by vitamin C (Schäffer, 1936).

7. Clinical studies on vitamin C and hyperthyroidism. According to Löhr (1936) vitamin C will reduce the blood iodine in normal and hyperthyroid patients. He reports clinical improvement in Graves' disease, but often without the B. M. R. being altered. In 1938 Eitel observed a latent vitamin C deficiency in patients with hyperthyroidism. He concluded that the administration of vitamin C to hyperthyroid patients had a symptomatic effect but not a direct action on thyroid activity. At the same time Thaddea and Runne (1938) showed

that hyperthyroid patients had a subnormal excretion of vitamin C after a test dose was administered, while two patients with myxedema excreted large quantities of vitamin C. This was confirmed by Lewis (1938) who found that the vitamin C excretion of 5 hyperthyroid patients receiving a constant diet was below normal, but rose to normal in 4 of the 5 cases after thyroidectomy.

Summary. Vitamin C deficiency produces hemorrhagic infiltration and hyperplasia of the thyroid gland of scorbutic guinea pigs, the effect being more marked in chronic than acute scurvy. Experimental hyperthyroidism increases the requirements for vitamin C. The effect of vitamin C on the liver glycogen of hyperthyroid animals is not definite, but vitamin C will reduce the creatinuria found in hyperthyroid animals. Hyperthyroidism will also reduce the amount of tissue vitamin C. Vitamin C will partially reduce the B. M. R. of hyperthyroid animals, but its effect on thyroid stimulation produced by the thyrotropic hormone is still controversial. Clinical studies have also shown an increased requirement for vitamin C in hyperthyroidism.

THYROID GLAND AND VITAMIN D. *1. Effect of vitamin D deficiency on the thyroid gland.* In early studies on rickets thyroid hypertrophy and hyperplasia was observed (Mellanby and Mellanby, 1921a; Murray, 1923). Rats maintained on the Steenbock rachitogenic diet also show thyroid hypertrophy and hyperplasia, but the changes produced developed in the presence or absence of vitamin D (Clausen, 1928; Krause and Monroe, 1930; Thompson, 1932; Levine, Remington and v. Kolnitz, 1933). The latter authors definitely showed that the changes produced in the thyroid by Steenbock's rachitogenic diet were due to a deficiency of iodine. The administration of cod liver oil to such rats may supply the daily requirement of iodine. In a later report Remington and Levine (1936) found no effect of the presence or absence of vitamin D on the degree of goiter produced in rats. Drennan, Malcolm and Cox (1931) and Sure (1938) did not observe any effect of vitamin D deficiency on thyroid size. Changes in the thyroid gland reported by other authors (Nitschke, 1933; Bennholdt-Thompson and Wellman, 1934; Uotila, 1938) can be regarded as due to imbalanced diet. Similarly, the decrease of iodine in the thyroid gland during vitamin D deficiency is due to a low intake of iodine (Krause and Monroe, 1930; Levine, Remington and v. Kolnitz, 1933).

Although human rickets is said to reduce blood iodine (Nitschke and Doering, 1933; Nitschke, 1934) this observarion has not been confirmed (Fasold, 1934a, b; Toepfer, 1934a, b). According to Kunde and Williams (1926) rabbits thyroidectomized soon after birth developed changes in the bones resembling rickets.

2. Effect of vitamin D administration on the thyroid gland. Large doses of irradiated ergosterol were found to increase the B. M. R. (Reed, Thacker, Dillman and Welch, 1933; Nitschke, 1933; Reed, 1934). Elmer, Geidosz and Scheps (1935) did not observe any effect of vitamin D on the thyroid of guinea pigs. However, Lands and Stoland (1935) observed that while non toxic amounts of irradiated ergosterol had no effect on the thyroid of cats, larger doses increased the amount of colloid. Similar results were obtained by Nitzesco and Bratiano (1936), Bruman and Bromberg (1936), Kucera and Soos (1933) and Goormagh-

tigh and Handovsky (1935). Handovsky and Goormaghtigh (1937) reported that 40 to 60 gamma of vitamin D_2 will produce some increase in thyroid function in dogs, and that with 110 to 230 gamma per day the thyroid was histologically hyperplastic. Thyroparathyroidectomized dogs tolerated 600 gamma per day very well, whereas thyroidectomized dogs tolerated this dose very badly with severe damage to the aorta and large arteries. They concluded that the action of the thyroid tends to protect the aorta and large arteries, while the action of the parathyroid gland intensifies the injurious action of excessive vitamin D.

As parathyroid extract did not increase the B. M. R. of dogs, Steck, Reed and Miller (1934) decided that vitamin D did not produce its effect by this means. Later Deutsch, Reed and Struck (1936) administered vitamin D to 2 thyroparathyroidectomized dogs, and did not obtain any increase in metabolic rate as they had in normal dogs. Thus the effect of large doses of vitamin D on the metabolic rate depends on an intact thyroid gland. Unpublished results have shown that the calorigenic effect of vitamin D is mediated through the thyrotropic hormone of the anterior pituitary gland (cf. Reed, Struck and Steck, 1939).

Vitamin D has also been reported to stimulate the oxygen consumption of frog muscle (Gelfan, 1935). Elmer, Giedosz and Scheps (1935) found no effect of vitamin D on thyrotropic stimulation of the thyroid gland, and vitamin D did not produce involution of the thyroid of rabbits fed a cabbage diet (Spencer, 1934).

3. Experimental hyperthyroidism and vitamin D. It has been shown that the calcium balance during hyperthyroidism may become negative due to a large increase in fecal calcium. The administration of vitamin D to normal animals will decrease fecal calcium and simultaneously increase urinary calcium excretion, suggesting that the effect of vitamin D on calcium metabolism during hyperthyroidism should be studied. Thus Taylor and Weld (1932) reported that vitamin D decreased the fecal calcium output of hyperthyroid dogs and Pugsley and Anderson (1934a) obtained similar results on rats, although a positive calcium balance was not produced. It was then demonstrated that, with the proper dosage, vitamin D would produce a calcium balance in thyroid-fed rats (Pugsley and Anderson, 1934b).

4. Clinical studies on vitamin D and hyperthyroidism. Tibbets, McLean and Aub (1932) found no effect of vitamin D on calcium metabolism during hyperthyroidism, whereas Hansman and Wilson (1934) reported a beneficial effect of vitamin D on calcium balance.

Summary. A deficiency of vitamin D has no effect upon the thyroid gland. The administration of vitamin D to normal animals increases the B. M. R., probably through its effect on the thyroid gland. When given in experimental hyperthyroidism, vitamin D will counteract the increased calcium excretion, returning the calcium balance to normal. Further work is needed before the effect of vitamin D on calcium balance in Graves' disease can be ascertained.

THYROID GLAND AND VITAMIN E. *1. Effect of vitamin E deficiency on the*

thyroid gland. The reports of Paal and Kleine (1933), Singer (1936), Barrie (1937) and Bromskov and Schneider (1939) indicate that vitamin E deficiency in rats produces a picture of decreased thyroid activity. However Telford, Emerson and Evans (1938) did not find any evidence of thyroid hypoplasia in vitamin E deficient female rats, and Biddulph and Meyer (1941, 1942) reported an increase in thyroid activity during vitamin E deficiency. The latter authors found that vitamin E deficiency increased the thyroid weight and B. M. R. of male rats, producing no change in female rats. They also found that the E deficient animals were receiving only about one-fourth as much iodine as control rats on a stock diet. By the addition of iodine to the diet of E deficient males they were able to prevent the changes in the thyroid and the increase in B. M. R. from occurring. They conclude that the effect of vitamin E deficiency in increasing thyroid weight and the B. M. R. is due to a relative deficiency of iodine.

Bromskov and Schneider (1939) noted that the thyroid of vitamin E deficient rats resembled that of ovariectomized animals, and that the administration of follicular hormone restored the thyroid function of vitamin E deficient rats. Vitamin E would also restore the thyroid of the deficient animals to normal, but had no effect on the thyroid of deficient animals that were ovariectomized. They believe, therefore, that the effect of vitamin E on the thyroid is mediated through the ovary.

The administration of vitamin E had no effect on the thyroid of normal rats (Bromskov and Schneider, 1939) or guinea pigs (Giedosz, 1938c), although Paal and Kleine (1933) reported stimulation.

2. Thyrotropic hormone and vitamin E. Bromskov and Schneider (1939) reported that the administration of thyrotropic hormone would restore the thyroid of their vitamin E deficient animals to normal. They also found no reduction in the thyrotropic content of the pituitary of E deficient animals, and no change in the antithyrotropic function of the blood of such rats.

Summary. Further experiments are necessary to ascertain if the changes reported to occur in the thyroid gland during vitamin E deficiency are a result of this deficiency or are due to other dietary factors.

GENERAL SUMMARY

Certain general statements on the interrelations between thyroid function and vitamin metabolism may be made. While some vitamin deficiencies will affect the thyroid gland, a number of the reports emphasize the necessity of controlling such factors as the length of time a deficient diet is fed, the influence of sex difference and whether or not the deficiency is acute or chronic. Each of these variables will influence the results obtained. Also, if a given vitamin deficiency will produce a picture of thyroid hypo- or hyperfunction, it does not follow that overdoses of the same vitamin will produce the opposite effect. Further work is also needed to ascertain the relation of the pituitary gland to the changes produced in the thyroid gland by a vitamin deficiency or the administration of a vitamin.

It has been demonstrated that the requirements for vitamins A, B and C are

increased during hyperthyroidism, and if these increased requirements are not met a relative deficiency of the vitamins will ensue. Such a vitamin deficiency may produce changes previously ascribed to an effect of hyperthyroidism per se. Whereas the administration of the necessary vitamins will correct these changes, it should not imply an antithyrogenic action, unless such an effect is demonstrated by further experiments. Conversely, if a vitamin will antagonize certain effects of thyroid feeding, it does not follow that a deficiency of the vitamin alone will increase thyroid activity. In studying vitamin metabolism during experimental hyperthyroidism there is also a sex difference in response, at least in the rat, and a difference in the degree of hyperthyroidism produced by the commonly used doses of thyroxin or thyroid gland. Also, growing rats may gain weight for a considerable period on the same dose of thyroxin or thyroid gland that will produce a progressive loss of weight in adult rats. These factors are of importance in studying the effect of a given vitamin on hyperthyroid animals.

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